ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE ON AGING
Fiscal Year 1981

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

NATIONAL INSTITUTE ON AGING

ANNUAL REPORT of program activities.

October 1, 1980 through September 30, 1981

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OFFICE OF THE DIRECTOR

In 1981, the National Institute on Aging continued to explore a wide range of medical and social issues affecting the nation's growing older population, from mandatory retirement of airline pilots to scientific progress in the treatment of Alzheimer's disease. Many of these topics were addressed at conferences and workshops organized or sponsored by the NIA, while others were the result of special studies commissioned by the Institute.

The Institute also continues to make organizational changes to provide a stronger administrative framework. A major advance made during FY81 was the formal establishment of the major extramural programs of the Institute: Biomedical Research and Clinical Medicine; Social and Behavioral Research; Epidemiology, Demography and Biometry; and Office of Planning and Extramural Affairs. Branches are now being formed in each of these programs.

New mechanisms were created for the recruitment and training of academic investigators in geriatrics, including a new small grants award planned for early 1982. In addition, work continues on the updated National Research Plan on Aging, which outlines priorities for biomedical and behavioral sciences research on aging.

Highlights of new projects, conferences, and program initiatives include the following:

Teaching Nursing Home

In April 1981 the NIA proposed the creation of teaching nursing homes, similar to teaching hospitals, to help the nation gear its biomedical and other research and training capacities to the needs of its growing elderly population. Recommendations were made to establish a public and private sector partnership to develop teaching nursing homes in affiliation with universities, especially their medical, nursing, and social service schools.

The nation's nursing-home spending is nearing $22 billion a year and probably will quadruple by 1990. Not enough is known about effective and cost-saving methods of diagnosis and treatment nor about ways to prevent illness or bring older people back to health. It would be to the advantage of both the public and private sectors to make sure that the large sums spent on long-term care are spent as effectively as possible.

The teaching nursing home would focus on such research topics as senile dementia, fecal and urinary incontinence, gait disorders, management of bedsores and pain, nutritional problems in healing and rehabilitation, and drug interactions.

In addition to conducting research, the model facility would be a place for training professionals for careers in research and service. Doctors, nurses, pharmacists, social workers, nurse aides, and physical, occupational, and speech therapists would have opportunities to work and learn collaboratively.

Funding mechanisms are being explored, and the Institute hopes to publish within the next year a program announcement on the establishment of teaching nursing homes in conjunction with university medical schools or medical centers.

National Research Plan on Aging

Public Law 93-296, which authorized the establishment of the NIA in 1974, also mandated that the Institute develop a research plan on aging. This plan, entitled Our Future Selves, was developed principally by the National Advisory Council on Aging, with staff assistance from the NIA, and was submitted to the Assistant Secretary for Health in 1976. By reviewing the state of aging research, by discussing priorities for scientific investigations in relation to the needs of the elderly population, and by outlining research opportunities in the mid-1970's, the plan was useful to scientists, administrators, policymakers, and the lay public.

An update of that plan is now in preparation by a panel of consultants to the NIA. Some of the objectives of this plan are: to provide a means for the Director, NIA, to inform the President, Congress, scientific community, and public concerning gains in our understanding of the aging process, the role of research, research progress to date, and future needs in aging research; to provide a stable, long-term framework for conducting research on aging within both the public and private sectors; and to identify opportunities in aging research which will attract scientists to the field.

Basic to the plan's development is the concept that an integrated approach combining biomedical, behavioral, and social research is most appropriate for achieving solutions to the broad range of concerns. The final plan is scheduled for completion in 1982.

Airline Pilots Study

In 1979 Congress mandated a study of the effect of aging on airline pilot health and performance, as a means of determining if mandatory retirement at age 60 is warranted. The Experienced Pilots Act requested that NIH undertake this study in conjunction with the Department of Transportation and submit a report of its findings to Congress. The legislation questioned the adequacy of medical certification procedures and examinations for commercial and airline transport pilots and, in particular, the "age 60 rule."

The NIA contracted with the Institute of Medicine of the National Academy of Sciences to obtain an objective review, summary, and assessment of existing scientific knowledge relevant to the questions posed in the legislation. A panel of experts was then formed to examine the IOM report, review public comments, and assist the NIH in preparing its response to Congress. The first meeting of the NIA panel was held on April 27-28, 1981. The panel's report will be sent to Congress on September 30, 1981

Program Announcements

A. Alzheimer's Disease and Other Related Dementias of Aging

Dementia affects 5 percent of the population over 65, or one million people. An additional 10 percent of the population shows varying degrees of cognitive dysfunction. Pathological studies indicate that at least 55 percent of persons dying with the diagnosis of dementia have Alzheimer's disease. A significant number of nursing home patients suffer from chronic dementias, and the current expenditure for nursing home care is approximately $22 billion per year. Even a 10 percent reduction in the number of patients with dementia would result in considerable savings annually. The enormous societal costs of this disease indicate the need for an increase in research efforts.

In May 1981 the NIA and the National Institute of Neurological and Communicative Disorders and Stroke jointly announced a continuing interest in research on Alzheimer's disease and other related dementias of aging. Research grant applications concerned with both fundamental and/or clinical investigations on the etiology, pathogenesis, diagnosis, treatment, and prevention of Alzheimer's disease are being sought.

B. Social and Behavioral Research on Aging

A revision of the NIA's program announcement in the area of social and behavioral research was published in May 1981. This announcement encourages the submission of grant applications designed to show how social, cultural, economic, and psychological factors operate to affect both the process of growing old and the place of older people in society.

C. Program Announcements in Preparation

Announcements on decubitus ulcers and urinary incontinence are in the preparatory stages and will be published in the NIH Guide to Grants and Contracts early in Fiscal Year 1982.

New Grant Mechanisms

A. Small Grant Program for Pilot Studies

The NIA is currently establishing a small grant mechanism to provide increased opportunities for investigators to conduct high-quality small-scale research on aging. One goal is to provide relatively rapid financial support, with an upper limit of $15,000 for up to a 12-month period. February 1, 1982 is the proposed application receipt date.

Research grant awards will be limited to four selected program areas: nutrition and health of the aged adult, pharmacology, senile dementia of the Alzheimer's type, and psychological studies on health and physical functioning.

B. NIA Academic Award

The purpose of the Academic Award Program is the recruitment and preparation of future academic investigators for careers in research and teaching, with special emphasis on geriatric medicine and related clinical disciplines. The award, made to an institution, provides a superior candidate with opportunity for 5 years of special study and supervised experience to further his/her individual needs.

The Academic Award is designed to provide support for individuals with high potential for academic and/or research careers in clinical areas. It bridges the gap between the initial period of postdoctoral study and a formal academic appointment. The Academic Award differs from the Individual National Research Service Award in that the Academic Award provides a continuing 5-year period of support for the future teacher-clinical researcher. This Academic Award differs from the Research Career Development Award in that it seeks to identify academic potential as differentiated from research achievement, and it emphasizes expertise in research, curriculum development, and teaching in clinical areas.

Candidates are restricted to those holding health-professional degrees in the clinical sciences (M.D., D.O., or equivalent). At the time of receipt of the award, the candidate must have completed at least 3 years of postdoctoral training and/or experience.

White House Conference on Aging (WHCoA)

The NIA continues to work closely with the staff of the WHCoA, scheduled for November 29 to December 4, 1981. Thirty-six scientific papers on various health topics were commissioned by the NIA for the conference. NIA staff members have prepared lay summaries of a number of these papers, which will be published by the WHCoA and distributed to delegates at the Conference. The NIA is also actively involved in the preparation of exhibits from each of the Institutes at NIH and will display its own large exhibit which will emphasize program priorities of the NIA.

In addition, the NIA has written two progress reports--one on senile dementia of the Alzheimer's type and the other on geriatric medicine--as well as two shorter brochures entitled "What is Aging Research?" and "What is Geriatric Medicine?" for distribution at the Conference.

A series of mini-White House Conferences have been held, three of which--on oral health care, impaired hearing, and low vision--were supported by the NIA. Along with the NINCDS, the NIMH, and the Alzheimer's Disease and Related Disorders Association, the NIA also sponsored a mini-conference on Alzheimer's disease and related disorders. This conference explored biochemical and pharmacological issues, risk factors, and social and psychological aspects of Alzheimer's disease.

Workshops and Conferences

A. Workshop on Urinary Incontinence

On November 3-4, 1980, the NIA brought together a small group of medical specialists and industry representatives to discuss urinary incontinence in the elderly. The purpose of the workshop was to define the incidence, causes, diagnosis, and treatments of this widespread problem, and to make recommendations for future studies.

The workshop was roughly divided into four areas: prevalence, etiology, and diagnosis; management techniques and treatments (including surgical, pharmacologic, and behavioral); attitudes and problems in long-term management, especially in institutions; and mechanical and other devices (absorbent materials, special clothing, etc.).

Among the elderly, urinary incontinence is one of the most common causes for admission to hospitals, along with impaired ambulation and dementia. In addition, up to 50 percent of those in skilled nursing homes probably suffer from some degree of urinary incontinence.

Incontinence is not a disease, but a physical sign of an underlying medical or psychological problem. In this country, urinary incontinence is both underdiagnosed and undertreated, and is largely a "hidden" condition. Too often, health-care providers (family members, nurses, or other nursing home personnel) view urinary incontinence as an unpleasant and demanding sanitation problem, while among elderly sufferers themselves the overriding emotional responses are frustration, embarrassment, and fear.

The workshop generated a sense of the priorities in research, giving those present a better sense of what types of studies are feasible and desirable. Also emphasized was the need to promote better research on the association of incontinence with specific diseases and the degree of idiopathic incontinence that cannot be traced to any specific disease process.

A technical paper based on the proceedings of this workshop has been prepared and will be submitted for publication this year.

B. Non-Lethal Biological Markers of Physiological Aging

As a first step toward assessing interventions--dietary, exercise, and other manipulations--that might alter the rate of human aging, the NIA held a conference on June 19-20, 1981, to evaluate the biological markers of aging.

"Biomarkers" of aging are those physical and behavioral changes occurring at predictable times during the aging process. Biomarkers can be used to measure and evaluate the effectiveness of interventions which may extend the vigorous and productive years of life.

Conferees discussed more than 20 possible biomarkers in animals and humans. To be useful, a biomarker must be reproducible, inexpensive, and consistently present in several species. In addition, the biomarker must change significantly with age and must indicate changes that take place within a short time frame.

In humans, some predictable aging markers include loss of specific hearing, vision, cardiovascular changes; bone loss; sleep variations; and alterations in glucose tolerance and immune function.

A marker which could be especially useful in human studies is forced vital capacity, a measurement of lung capacity and chest wall musculature. This test is an exceptionally good indicator of general health and vigor. Investigations have shown that forced vital capacity values fall at a rate of 3.8 deciliters per decade in men and 3.1 deciliters per decade in women.

These and other markers discussed at the conference were found to be good indicators of aging, as well as practical for use in intervention studies. Other markers must be eliminated for reasons such as unreliability, expense, and time factors. Conference proceedings will be published later this year.

C. International Developments in Aging

In conjunction with its regular meeting in May 1981, the National Advisory Council on Aging held a symposium on International Developments in Aging. Included for discussion were the designation of the NIA as a World Health Organization Collaborating Centre for Joint Cooperation on Research on the Care of the Aged, and the 1982 United Nations World Assembly on the Aging. Presentations were made by several representatives of the WHO and the UN. The purpose of the symposium was to inform participants of the activities in aging currently being conducted throughout the world.

D. Planning Workshop on the Neurochemistry of Alzheimer's Disease

At this NIA workshop held in August 1981, government scientists and representatives from some of the leading research institutions discussed a wide range of issues including: the etiology and pathogenesis of Alzheimer's disease; neuropharmacology and neurochemistry; diagnosis (clinical-pathological-biochemical correlations); and scientific and organizational issues influencing research on Alzheimer's disease.

E. Perspectives on Prevention and Treatment of Cancer in the Elderly

The second in a series of working conferences on aging and cancer was held in September 1981. It was jointly sponsored by the NIA and the NCI. Among the topics discussed were: pathophysiological and surgical considerations in caring for the elderly cancer patient; site-specific treatment perspectives (breast, colorectal, prostate, and skin cancer); and screening, early detection, and diagnosis. A major conference goal was the recommendation of research efforts in selected areas where knowledge is unavailable or ambiguous, and intervention techniques which merit additional exploration and development.

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INFORMATION OFFICE

The following list of achievements covers the period from September 4, 1980 to May 13, 1981.

Announcements/Press Releases

1. *Extramural Research and Training Program: Grant and Contract Summaries FY 1974-1977* (notice of availability)
2. *Perspectives on Geriatric Medicine/Observations on Geriatric Medicine* (publication announcement)
3. *Research Training Opportunities at the National Institute on Aging Gerontology Research Center* (publication announcement)
4. *Proceedings of the Second Conference on the Epidemiology of Aging* (publication announcement)
5. *Special Report on Aging 1980* (publication announcement)
6. *A Winter Hazard for the Old: Accidental Hypothermia* (publication announcement)
7. *Biochemistry of Aging* (notice of availability)
8. *A Guide to Medical Self-Care and Self-Help Groups for the Elderly* (publication announcement)
9. "Senility Reconsidered" press advisory
10. Appointment of Richard Suzman (never released)
11. Appointment of Richard Sprott (never released)
12. Appointment of Edward Schneider (never released)
13. Press Release on *A Treasure Hunt*
14. Statement on hyperthermia (for the Surgeon General)
15. Press advisory on Reichmann Memorial Lecture
16. NIA Grantee Reports Unsuspected Visual Handicap (prepared but not yet released)

Annual Reports

1. Annual Report (FY 80)--wrote Office of the Director and Information Office sections; compiled and processed entire NIA submission
2. NIH Almanac (FY 80)
3. Scientific Directory/Bibliography 1981 (twice yearly)
4. Special Report on Aging 1981
5. Developments in Aging 1981
6. Program Review (FY 80)
7. NIA Freedom of Information Report (FY 80)
8. NIA sections of Diabetes Special Report (1981)--editing, clearance, transmittal
9. NIA sections of Genetics Special Report (1981)--editing, clearance, transmittal
10. NIA section of NIH Publications List
11. Annual Public Affairs Report to HHS (FY 80)

Projects

COMPLETED:

1. Three videotapes for the NIA exhibit
   - --general audience
   - --scientific audience
   - --children

2. Interagency Agreement for preparation of lay publications for the White House Conference on Aging

3. Public awareness campaign on accidental hypothermia

4. Launching of the Aging Update series

5. New distribution channels for the Age Page and other NIA publications
   - --Consumer Information Center
   - --Supermarket Communication Systems
   - --Administration on Aging
   - --State and Area Agencies on Aging
   - --Organizations for the aged and ethnic groups
   - --Pharmacies

6. Research for and assistance on ten NBC programs on aging
   - --developed a quiz on basic aspects of aging
   - --provided slides and other background material
   - --wrote some dialogue
   - --assisted in production

7. Translation of two Age Pages (on accidents and minorities) and A Treasure Hunt into Spanish

8. Promotion of A Treasure Hunt
   - --worked with WDVM-TV to produce a special news segment
   - --special Christmas program at the Clinical Center children's ward
   - --contacts made with The New York Times, Today Show, Good Morning America

9. Obtained new equipment for existing NIA exhibit and supervised refurbishment of exhibit

10. Hall exhibit for second floor

11. Updated photograph and slide collections and developed annotated filing system for photographs, videotapes, and slides

12. Radio spot for accidental hypothermia brochure

13. Updated media contact list

14. Media relations with major networks--sent background and followed up with phone calls

15. Developed NIA logo

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16. Updated summary of NIA program announcements

17. NACA 5th Anniversary celebration wrap-up
    - --assembly of photographic archive
    - --preparation of festschrift
    - --striking of commemorative medallions

18. Assistance to OPEA in developing Sourcebook instructions

19. Assistance to SBR in clearance and publication procedures for a book, Leading Edges

20. Assistance to SBR in clearance and publication procedures for a book, Inventory of Longitudinal Studies on Aging

21. Assistance to OPEA in clearance and publication procedures for a book, The Quality of Life in the Later Years

22. Summary of research on abuse of elderly

23. Briefing on NIA SDAT activities for meeting with Secretary Harris

24. Assistance to CBS for presentation on "senility"

25. Reviewed Freese article for Better Homes and Gardens

26. Public awareness campaign on senile dementia of the Alzheimer's type

27. Became actively involved in NIH efforts to improve the mailing key system
    - --provided background to the Associate Director for Communications, NIA
    - --participated in testing of new procedures

28. Gathered data on diabetes in the elderly for Dr. Ringler

29. Routine updating of Dr. Butler's curriculum vitae and notification to selected organizations of recent awards

30. Took photos of Dr. Butler for media and other uses

31. Advance work for Dr. Butler's appearances
    - --Gerontological Society meeting
    - --Donahue show
    - --American Association of Homes for the Aged speech
    - --Frieda Fromm-Reichmann Memorial lecture

32. Preparation of materials on senile dementia for the Wall Street Journal

33. Participation in Undersecretary's Long-Term Care Task Force
    - --research and memoranda on HEW proposed regulations on nursing homes

34. Preparation of portable exhibit for the NIA EEO Office

35. Responded to numerous Congressional inquiries

36. Updated NIA publications list

37. Organized press clippings into subject file system

38. Production assistance for WDVM-TV segment on the longitudinal study

IN PROCESS:

1. Film series and other media activities for the White House Conference on Aging

2. Bibliographies/reading lists
   --general readings on aging
   --bibliographies and information sources on aging
   --organizations concerned with aging
   --university centers in gerontology
   --journals in the field of aging
   --geriatric medicine
   --retirement
   --older women
   --sexuality and aging

3. Assistance to Dr. Butler in guest editing a special issue of Generations magazine on sexuality and aging

4. Assistance with Pilots Study

5. Public Service Announcements to promote the Age Page and A Treasure Hunt (holding until funds permit)

6. Translation of additional--current and future--Age Pages into Spanish (holding until funds permit)

7. Updating demographic charts for lay audiences and producing a demographic fact sheet on aging

8. Revision of NIA orientation videotape

9. Development of aging curriculum for junior high students (holding until funds permit)

10. Compilation and translation of classic cases in aging

11. System for designating historical publications

12. Recompetition of NIA addressing and mailing contract

13. Draft interim report on national plan for research on aging

14. Participation in committee to develop policy on NIA-AoA interface

15. Participation in committee to develop policy on teaching nursing home

16. Guidelines for NIA staff in handling press contacts

Brochures

COMPLETED:

1. Biochemistry of Aging--summary of a workshop held in January 1978 on prospects for research in the biochemistry of aging.

2. Perspectives on Geriatric Medicine--interviews with leaders in the field of geriatrics on the increasing need for medical training in the health care of older Americans.

3. Special Report on Aging 1981--summary of NIA intermural and extramural research highlights and program accomplishments during FY 1980.

4. What Is Aging Research?--fact sheet prepared for initial distribution to delegates and attendees at the White House Conference on Aging. (not yet published)

5. What Is Geriatric Medicine?--fact sheet prepared for initial distribution to delegates and attendees at the White House Conference on Aging. (not yet published)

6. Progress Report on Geriatric Medicine--a summary of recent developments in the rapidly evolving field of geriatric medicine. (not yet published)

7. Special Report on Senile Dementia of the Alzheimer's Type--highlights recent dramatic findings in the study of senile dementia, many of which have sparked increased scientific interest as well as public awareness that there is hope for the elderly of the future. (not yet published)

8. A Winter Hazard for the Old: Accidental Hypothermia--a revision and redesign of the brochure that warns the elderly to protect themselves against this progressive drop in deep body temperature that can be fatal if it is not detected in time and treated properly.

9. A Guide to Medical Self-Care and Self-Help Groups for the Elderly--an update of the popular guide to self-care and self-help organizations with special interest in or relevance to the elderly.

REPRINTED:

1. Changes: Research on Aging and the Aged

2. A Treasure Hunt

3. To Understand the Aging Process: The Baltimore Longitudinal Study of Aging

4. The Older Woman: Continuities and Discontinuities

5. Q & A: Alzheimer's Disease

IN PROCESS:

1. Festschrift of NACA 5th Anniversary Meeting
2. Menopause and Aging
3. New NIA brochure
4. Proceedings of the Conference on Exercise in the Elderly
5. Age Words: A Glossary of Gerontology
6. Skin Aging
7. The Older Woman
8. Geriatric Care Series (3 publications)
9. Lay summaries of technical papers for the White House Conference on Aging
10. Baltimore Longitudinal Study of Aging book
11. Revision of Welcome to the NIA brochure
12. Facts and Figures About Aging in America

Age Pages

COMPLETED:

1. Heat, Cold, and Being Old
2. What To Do About Flu
3. Skin: Getting the Wrinkles Out of Aging
4. Safe Use of Medicines by Older People
5. Las Minorias y Como Ellos Envejecen
6. Los Accidentes y Los Ancianos

IN PROCESS:

1. When an Older Husband or Wife Dies
2. Taking Care of Your Teeth
3. Estrogens: How Does a Woman Decide?
4. Foot Care for Older People
5. Aging Quiz

6. Bereavement
7. Stress
8. Retirement
9. Sex
10. Definitions of Health Professionals
11. Preventive Care
12. Myths About Aging
13. Safety Tips for Urban Elderly
14. Pain
15. Exercise
16. Memory

Aging Updates

COMPLETED:

1. The Teaching Nursing Home

IN PROCESS:

1. The Poverty of Poverty Statistics
2. Incontinence

Articles/Speeches

1. The Teaching Nursing Home (JAMA)
2. Opening Remarks (International Study Group on Pharmacology of Memory Disorders)
3. The Iron Lung of Geriatrics (American Association of Homes for the Aged)
4. The Importance of Research in the Field of Aging (edited for future publication)
5. Pharmacy's Contribution to Geriatric Care (Drug Intelligence and Clinical Pharmacy)
6. Preface to The Myth of Senility
7. Preface to Brain Failure
8. Research--the Ultimate Service (Geriatric Nursing)
9. Foreword for Animals, Aging, and the Aged
10. Geriatrics and Manpower (Connecticut Medicine)
11. The Poverty of Poverty Statistics (Newsweek)
12. Editorial (Newsletter of the Alzheimer's Disease and Related Disorders Association)
13. Abstract of "Senility Reconsidered" (international issue of Interhaus Abstracts)
14. Article for University of Philadelphia Center for the Study of Aging newsletter
15. Preface to Elderly Patients and their Doctors
16. Preface to the Psychiatric Clinics of North America--Aging
17. Bringing Old and Young Together and Improving Life for Both (Hospital Progress)
18. Article on A Treasure Hunt (NIH Alumni News)
19. Cardiac Mortality Rates (edited EDBP article for JAMA)
20. Letters to "Dear Abby"
21. Changes in Biological Rhythms May Cause Sleep Disorders (JAMA)
22. Article on Biophysiology and Pathobiology of Aging Program (American Academy of Orthopaedic Surgeons magazine)

23. Students and the Elderly (The Synergist)

24. Caring for an Older America: The Role of Pharmacy (Pharmacy Times)

25. Statement for Dr. Butler at the White House Mini-Conference on Senile Dementia of the Alzheimer's Type

26. Senility (Search for Health column)

27. Patients Needed for NIH Studies (The Advocate)

28. Nutrition (Search for Health column)

29. An Approach to Estimating the Average Length of Stay of the National Nursing Home Population (edited for publication by EDBP)

30. Research Epidemiology as a Growth Industry at the National Institute on Aging (edited for EDBP submission to Public Health Reports)

31. Talking points for speech on women and aging

32. Accidents (Search for Health column)

33. Safe Use of Medicines by Older People (Search for Health column)

34. Nutrition: Staying Healthy in the Later Years (Appetizingly Yours)

IN PROCESS:

1. Essay for American Association of Geriatric Psychiatry newsletter on psychiatric training

2. Speech on "The New Gerontology"

3. Article for Revista Costarricense de Ciencias Medicas

4. Edited version of Dr. Butler's presentation at the FDA Bureau of Drugs Geriatric Symposium (to be published in Psychopharmacology Bulletin)

5. Safe Use of Medicine (for the National Council on the Aging News Service)

6. Editing Foreword and Chapter 12 of Leading Edges

7. Article for Counseling Psychologist

8. Chapter for Alzheimer's Disease and Senile Dementia

NIH Record Stories

COMPLETED:

1. Young and Old Meet in Triple Celebration at Capital Children's Museum
2. Research Frontiers in Aging and Cancer Symposium
3. Dr. Brody Most Highly Decorated Member of PHS
4. NIA Reorganizes
5. NIA Designated a WHO Collaborating Centre
6. New NACA Members (Carrozza and Schoenrich)
7. Dr. Butler to Speak at Frieda Fromm-Reichman Memorial Lecture
8. International Meeting on Aging to be Hosted by NIA

IN PROCESS:

1. Cell Culture Studies Yield Information on Human Aging

Conferences Covered

1. Research Frontiers in Aging and Cancer
2. NIH Consensus Conference on Cervical Cancer Screening
3. Workshop on DHEA and Dietary Restriction
4. Geriatric Medicine Academic Awardees' meeting
5. AARP Energy and the Elderly Consortium
6. National Research on Aging Planning Panel Meeting
7. NIA Workshop on Urinary Incontinence
8. White House Mini-Conference on Alzheimer's Disease and Related Disorders
9. White House Mini-Media Conference "Challenging Age Stereotypes in the Media"
10. Workshop on Age and Anthropological Theory

Conferences, Workshops, and Seminars Held, and Proceedings Published During FY 1981

The Information Office did not hold any conferences, workshops, or seminars during FY 1981. It did, however, assist in publicizing conferences sponsored by other NIA components and facilitated the publication of several conference proceedings, including:

1. Biochemistry of Aging
2. Biological Mechanisms in Aging
3. Nutritional Needs and Health of the Aging Adult

Staff Accomplishments and Activities in FY 1981

A. Publications

All articles prepared or edited by the Information Office were for the signature of the Director, NIA or were given anonymous attribution to the Institute.

B. Presentations

1. Jane E. Shure gave a speech about the health problems of older women at the Montgomery County Women's Fair.

2. Clarissa Wittenberg spoke to the Montgomery County Provider Council about research on aging.

C. Awards and Other Forms of Recognition

1. The Public Service Announcement "Don't Put Older People on the Shelf" won a bronze medal in the International TV and Film Critics Awards and a second place award from the National Association of Government Communicators.

2. Ruth Dubois received a quality increase.

3. Jeanne Abrahamsson received a quality increase.

D. Other Significant Activities

1. Staff Training
   --Seminar on Social Marketing (Ann Dieffenbach, Marian Emr)
   --EEO Training (all Information Office staff)
   --Capitol Hill Workshop (Ann Dieffenbach)
   --Freedom of Information Workshops (Esther Solomon)
   --Psychiatric Treatment Issues in the Elderly (Marian Emr)
   --Public Relations in Washington (Clarissa Wittenberg)

2. Committee Appointments
   --NIA EEO Committee (Ann Dieffenbach)
   --NIH Printing Liaison Committee (Ann Dieffenbach)
   --Several NIH QRB's

3. The Age Page--a series of one-page health information sheets for older persons, their families, and those who work with the elderly--continues to be a successful and popular publication. Since April 1980, 10 issues have been published:

   Finding Good Medical Care for Older Americans
   Accidents and the Elderly
   Minorities and How They Grow Old
   High Blood Pressure: A Common But Controllable Disorder
   Senility: Myth or Madness?
   Safe Use of Medicines By Older People

Food: Staying Healthy After 65
What to do About Flu
Heat, Cold, and Being Old
Skin: Getting the Wrinkles Out of Aging

The edition on nutrition has been especially popular and was used as the basis for newspaper articles in over 50 communities in the United States. One syndicated column generated over 2,500 requests for additional nutrition information in a 2-month period.

In an article on senile dementia, Better Homes and Gardens magazine mentioned the availability of our Age Pages on Finding Good Medical Care and Senility. This single article generated approximately 15,000 requests for these two issues.

The High Blood Pressure issue was also popular, possibly because of the large network of blood pressure screening centers in this country, many of which requested multiple copies for their patients.

We are making many efforts to reduce costs connected with the production and distribution of the Age Page. Because mailing represents the single most costly element, most of our efforts are in this area. In February 1981, two editions were mailed together, cutting in half the normal mailing costs for two issues. In the future (and as appropriate), we plan to present two topics in a single 4-page format, which will reduce both printing and mailing costs.

Bulk mailing rates are considerably lower than single-copy rates. When organizations such as senior centers, retirement communities, and nursing homes request permission to announce the availability of the Age Page in a newsletter, we suggest that they determine the number of interested individuals, which allows us to send them one bulk shipment instead of responding to many single requests. When large numbers are needed (over 200), we now request that organizations make their own reproductions (the Age Page has no copyright and thus no permission is required to reprint it).

We have recently established a system of inexpensively distributing large quantities of the Age Page in chain drug stores throughout the country. The National Association of Chain Drug Stores, with over 225 member organizations representing approximately 15,000 individual stores, has aided us in contacting their members with a proposal that we send each member 50 copies of all future Age Pages for each of their individual stores. The stores will display the Age Pages in their public information racks, near bulletin boards, or on the pharmacist's counter. So far, 65 percent of the chains have responded requesting copies for their stores.

Two of the Age Pages--Accidents and Minorities--have been translated into Spanish and will be printed early next fiscal year. We are developing a list of organizations that have either requested Spanish language materials or have a large number of Spanish-speaking members. One organization, the Asociacion Nacional pro Persones Mayores, has agreed to mail single and multiple copies of all Spanish Age Pages using their own mailing lists.

Topics to be covered in the next year include: sexuality, dental care, estrogen use, care of the feet, preventive medicine, and memory. We continue to receive many suggestions for new topics from our readers, and the overwhelming majority of our mail is positive.

4. The Information Office is currently arranging to show movies featuring masterpiece performances by older actresses or actors--or directed by older directors--in the evenings during the White House Conference on Aging. Panel discussions may also be held following the films. The experience gained in this project could be used to formulate an article on mature creativity, and eventually form the basis for a television program on the same subject.

5. Approximate number of inquiries answered:

   Letters and publication requests--approximately 130,000 per year

   Telephone calls--approximately 25,000 per year

   Age Page requests (since October 1980)--16,000*

*NOTE: Most requests were for a supply of Age Pages for further distribution

Social and Behavioral Research Program

Program Description SBR-1

Program Highlights SBR-1

SOCIAL AND BEHAVIORAL RESEARCH

PROGRAM DESCRIPTION

The Social and Behavioral Research (SBR) program has an all-embracing framework divided into four program areas, within which more narrowly focused initiatives are periodically defined. During 1981, SBR staff has actively pursued the development of grants in each of the four program areas, sharpened the focus of its initiatives, developed its points of convergence with a range of other programs, and engaged in a wide variety of developmental activities which will be briefly outlined here. The four program areas as defined a year ago are:

- Cognitive and Biopyschological Aging - concerned with the identification and specification of cognitive, intellectual, and perceptual changes and stabilities that, interacting with biological processes, occur with aging.

- Social Psychological Aging - concerned with changes and stabilities with aging in the health, personality, behavior, and attitudes of individuals as related to the social environment.

- Older People in the Changing Society - concerned with the changing societal and cultural conditions that affect people in their middle and later years in respect to mortality, the burden of illness, socioeconomic status, needs and attitudes, and the opportunity to lead productive lives.

- Older People and Social Institutions - concerned with how, and with what consequences, people are influenced by the intricate network of institutions in which they grow old: families, households, work organizations, educational and religious households, work organizations, educational and religious institutions, political institutions, health and welfare institutions (including nursing homes and community support systems.)

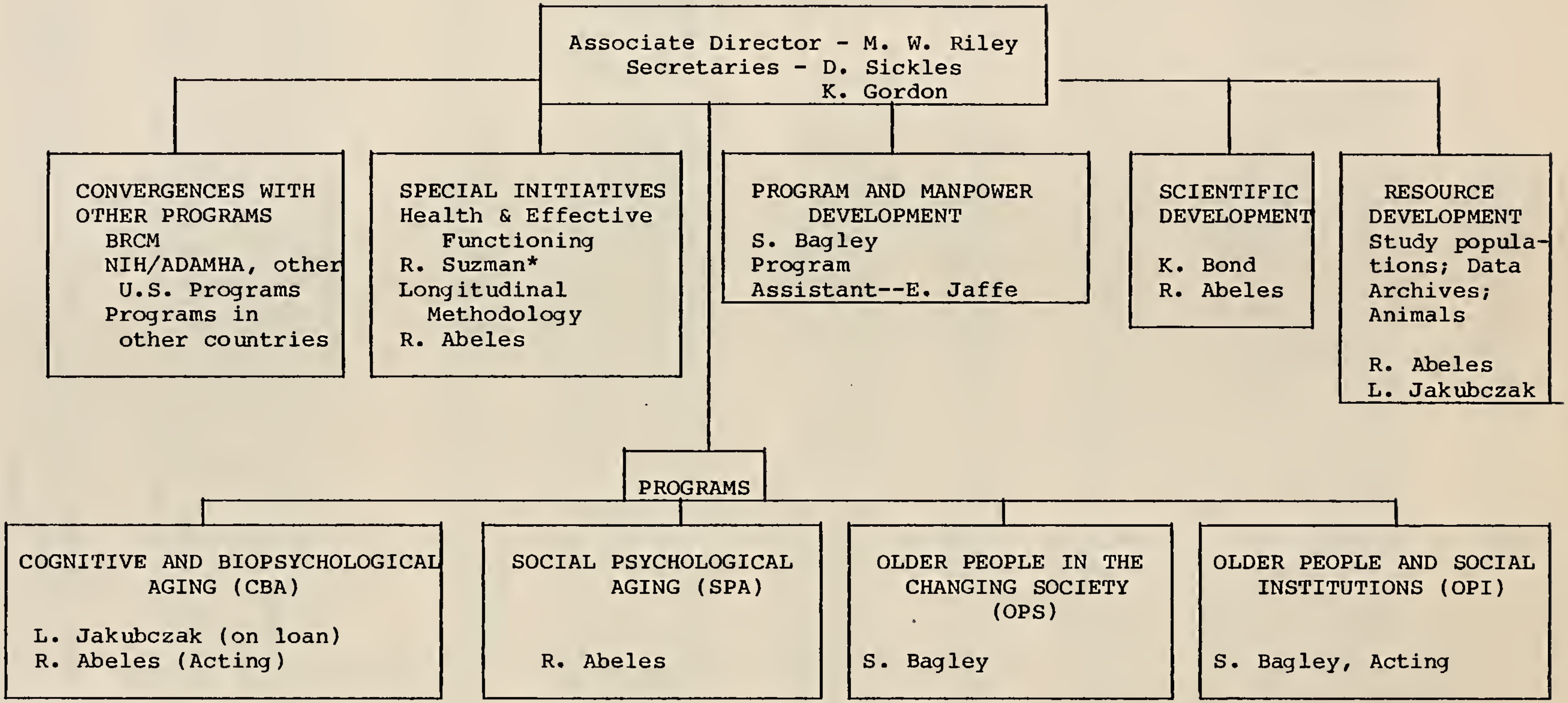
Figure 1 illustrates the current structure of SBR and the deployment of its personnel, indicating these four program areas and other major SBR functions.

PROGRAM HIGHLIGHTS

This is a propitious moment in the development of social and behavioral research on aging. SBR stands at the confluence of two significant intellectual developments, which have gradually introduced entirely new paradigms for research. The two developments are highlighted this year by the completion of the Duke Longitudinal Studies (1955-1980) and by the series of cohort-longitudinal studies at the University of Southern California (1956-1980). These studies underscore two principles, now widely accepted, that underlie the SBR program.

- First, aging changes with social change. The recognition that people in different cohorts age in different ways leads to research on how particular social and historical conditions influence aging.

SOCIAL AND BEHAVIORAL RESEARCH STRUCTURE AND ORGANIZATION


Associate Director - M. W. Riley
Secretaries - D. Sickles
K. Gordon
CONVERGENCES WITH OTHER PROGRAMS
BRCM
NIH/ADAMHA, other U.S. Programs
Programs in other countries
SPECIAL INITIATIVES
Health & Effective Functioning
R. Suzman*
Longitudinal Methodology
R. Abeles
PROGRAM AND MANPOWER DEVELOPMENT
S. Bagley
Program Assistant--E. Jaffe
SCIENTIFIC DEVELOPMENT
K. Bond
R. Abeles
RESOURCE DEVELOPMENT
Study populations; Data Archives; Animals
R. Abeles
L. Jakubczak
PROGRAMS
COGNITIVE AND BIOPSYCHOLOGICAL AGING (CBA)
L. Jakubczak (on loan)
R. Abeles (Acting)
SOCIAL PSYCHOLOGICAL AGING (SPA)
R. Abeles
OLDER PEOPLE IN THE CHANGING SOCIETY (OPS)
S. Bagley
OLDER PEOPLE AND SOCIAL INSTITUTIONS (OPI)
S. Bagley, Acting

Sections

Cognitive Aging
  L. Jakubczak
Biopsychological Aging
  (   )**

Support Staff: NONE

Support Staff: NONE

Sections

Age in the Population
  S. Bagley
Age and Social Structure
  R. Suzman*

Support Staff: Patricia King

Figure 1

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* I.P.A. - leaving October, 1981
** Position vacated by Richard Sprott

- Second, aging consists, not only of biological, but also of psychological and social processes. The recognition of the importance of the brain to the endocrine, immune, and other physiological systems - themselves changing with age - leads to research on accumulating behaviors and social stresses which feed through the brain and the mind to affect these systems.

These two major contributions are already resulting in behavioral and social research of basic significance. Behavioral and social research has demonstrated that many stereotypes of inevitable deterioration with aging are merely fallacious interpretations of cohort differences; that human competence in fact extends far beyond the average age of retirement; that intellectual and physical declines even at the oldest ages can often be reversed; that the functioning of old people in nursing homes can often be restored.

Such intellectual developments are taking shape at a strategic time - at a time when the Surgeon General reports that over half the deaths in the United States are attributable to behavior and life style; at a time when prevention and self-care are becoming the watchwords; at a time when the disabilities, dependencies, and institutionalization of old people must be forestalled before they become overwhelming.

A major objective of SBR is to provide the knowledge base for prevention and postponement of disability and dependence, and for enhancement of health and effective functioning in the middle and later years. While the potential is enormous, SBR has been working against two considerable odds. First, only limited grant funds have been available to support many applications which promise new and deeper understandings. Second, only limited staff and time have been available to stimulate and guide development of this potential in the scientific community.

Despite these limitations, 1981 has been a year of excitement, and optimism. SBR has persisted in its goal of understanding the social, cultural, economic, and psychological factors that affect both the process of growing old and the place of older people in society. By small increments, research continues to demonstrate the power of these factors, and to probe the linkages between the changing social environment and such potentially preventable or reversible disabilities of middle and old age as chronic ill health, sensory decrements, memory loss, low self-esteem, or exclusion from active participation in social and economic roles.

MAJOR PROGRAM AREAS AND INITIATIVES

Research and Training Grants

From the reorganization of SBR in Fiscal Year 1980, the parameters of the program areas were defined broadly with a purpose: not only do they establish a blueprint for the future, but more immediately they aim to attract top-flight scientists to aging research, thereby raising the level of excellence of NIA's contributions to the complex mosaic of accumulating knowledge in this large area.

Staff efforts during 1981 are notably successful in initial progress toward this immediate goal of enhancing the quality, if not the size of the program. Peer review groups increasingly give top priority scores to SBR applications. Several distinguished scientists, seeing the relevance of aging to their own work, have been added to the roster of grantees (among them Featherman and Sorensen, Wisconsin, on relating changes in aging to societal change; Converse, Michigan, on social support as it contributes to well-beling through the life course; Rodin, Yale, on dietary habits, stress, and health in later life; Baron, Wisconsin, on performance of older adults under time pressure). Nearly half the projects funded in 1981 were from investigators new to aging.

Also added to the roster this year are a number of promising young scholars (such as Cohen from the University of California at San Francisco on old people's means of coping with arthritis; Krauss from the University of Southern California on job conditions under which the performance of not-yet retired older workers matches their ability; Mayeux from Columbia on depression and Parkinson's disease.)

However, SBR efforts have been less successful in increasing the size of the program during 1981. Although firm comparative figures are not available, the drop from 1980 to 1981 is approximately 135 to 126 in total number of projects and from 10.5 million to 9.9 million in dollars. (The drop, while especially pronounced in training grants, is fairly even across the four program areas). The failure to grow is attributable to several factors, including the impossibility, because of fiscal constraints, to fund many superior projects despite priority scores clearly fundable in previous years; the dearth of training funds (although in the present state of the art. postdoctoral fellowships in interdisciplinary settings seem most needed); the crowded space in which staff must work and receive visiting scientists; the lack of funds for travel to research centers expressing interest; and above all, the shortage of staff to work with applicants.

The size of the grant program is definitely not restricted by lack of interest: responses to program announcements arrive daily, HSAs talk by telephone with several applicants every day, and the number of applications submitted exceeds the number of funded projects by some 7 to 1. The potential is there; but it must be cultivated.

SBR Initiative in Longitudinal Methodology

Of the several initiatives proposed a year ago, two have taken firm shape during 1981. One of these is the effort to improve and apply methods for longitudinal and cohort analysis. The central importance to aging research of longitudinal methodology is symbolized by NIA's receipt of the final report of The Duke Longitudinal Studies, 1955-1980, funded by NIA and its predecessor in NICHD. This report, a summation of the several hundred previously published articles and books, demonstrates more clearly than many of its counterpart studies two fundamental principles: that longitudinal studies are a unique means of isolating changes and stabilities in the aging process; and that biological, psychological, and social aging processes interpenetrate in such complex ways that no single process can be fully understood apart from the others. The Duke project has added importantly to the techniques and procedures for longitudinal analysis, laying the groundwork for such current SBR activities as: continuing support of the methodological and conceptual efforts of the Social Science Research Council's Committee on Life-Course Perspectives on Middle and Old Age; the explorations with NHLBI for possible analysis of data from the Framingham Heart Study in order to test and improve existing methods; and consideration of plans for an NIA directory of methodological consultants to improve the quality of grant applications. The social sciences have much to offer aging research in general in the way of sophisticated procedures for longitudinal and causal analysis, and for avoiding many fallacious interpretations pervading the current scientific literature. But much remains to be implemented through specific plans for the 1982-1985 agenda.

SBR Initiative in Health Promotion

Of the other initiatives planned a year ago, the one then called Psychosocial Components of Health Promotion has now been given depth and focus, and has been translated into the new SBR emphasis on Health and Effective Functioning in the Middle and Later Years. In focusing this initiative, the SBR staff has been heavily engaged in intellectual, developmental, and planning activities. Since this new initiative constitutes the second major line of SBR development during 1981, and prepares the way for many of the plans for 1982-1985, it is described in a separate section.

Developmental Activities

In order to develop its four program areas and special initiatives within the severe 1981 constraints of time and money, SBR staff has emphasized cost-effective activities such as the following:

- Grant mechanisms and Grantees - Revision of the broad program announcement on Social and Behavioral Research at NIA; preparation of the new program announcement on Health and Effective Functioning in the Middle and Later Years; refinement of referral guidelines for use by the Division of Research Grants; initial development of a small grants mechanism; investigation, and postponement because of current funding shortages, of the plan for an Interdisciplinary Research Award; advising and assisting applicants and reviewing advance drafts of proposals.

- Development of resources for the aid of grantees - Support of archives of computerized data for secondary analysis; support of longitudinal study populations from which new information may be sought; support of a sorely needed Inventory of Longitudinal Studies of Aging, to be published and distributed by NIA; development for psychosocial research of colonies of aging rodents and nonhuman primates; cooperation with BRCM in the animal models contracts program.

- NIA workshops and seminars for conceptual clarification and specification of research objectives - Conduct of a series of NIA symposia and seminars (by necessity scheduled without cost to the

Institute); convocation of a workshop of a dozen leading anthropological theorists, whose papers on the social and cultural variability of aging will be published as a book.

- Communication to the scientific community - Staff attendance at scientific meetings; staff presentations of well over two dozen scientific papers and descriptions of the NIA programs; staff publication of a long list of journal articles and books; SBR planning and commissioning of essays for Leading Edges: Recent Research on Social and Psychological Aging, a book prepared by NIA for the White House Conference on Aging; support of the three books prepared by the National Academy of Sciences Committee on Aging, scheduled for fall 1981, published by Academic Press (Stability and Change in the Family, The Elderly of the Future, Interactions of Biology and Behavior in Aging); and for future publications, staff planning of a wide range of symposia, and scientific presentations.

Coordination with Other Programs

A major mandate of the Associate Director, SBR is to evolve coherent directions and emphases from the many programs in aging research, not only within NIA, but also in the far-flung activities of other agencies both in the United States and abroad. During 1981, SBR staff has exerted great effort to consult, cooperate with, and participate in the related work in this country of organizations such as: the other National Institutes of Health; the Alcohol, Drug Abuse, and Mental Health Administration; the Administration on Aging; the task force of Federal agencies concerned with economics of aging (convened by SBR); the Institute of Medicine of the National Academy of Sciences; the American Association for the Advancement of Science; the Social Science Research Council; the American Psychological Association, the American Sociological Association, and other professional societies; a number of mini-White House Conferences on Aging; and several foundations and large corporations in the private sector.

Relationships to programs in other countries are especially important to SBR in view of its concern with understanding the influence of social and cultural conditions on the aging process, the lives of older people, and the general significance of age in society. During 1981, transnational relationships have been advanced by SBR through participation in: the North American Regional Technical Meeting in preparation for the 1982 World Assembly on Aging; the Heidelberg international meeting of the Social Science Research Council's Committee on Life-Course Perspectives on Middle and Old Age; the Nijmegen (the Netherlands) Workshop on Lifespan Development; the Hamburg meetings of the International Gerontological Association; and - with special reference to Communist countries - the Bellagio Conference on Retirement and Aging in Cross-Cultural Perspective, and the visits by NIA staff to gerontological centers in the German Democratic Republic.

Many of these program-wide activities have contributed directly or indirectly to the evolving new focus on Health and Effective Functioning in the Middle and Later Years.

New Focus: Health and Effective Functioning in the Middle and Later Years

SBR has set the stage for its future activities by a program announcement (issed in September 1981) on Health and Effective Functioning in the Middle and Later Years. This program announcement, in which BRCM is cooperating, invites qualified researchers to submit grant applications to NIA for research projects designed to specify in detail how social and behavioral processes, interacting with biological processes, influence particular aspects of health and functioning in the middle and later years.

This new emphasis springs from the 20th century triumph of extension of life, which means not only that the numbers of older people are increasing, but also that more and more individuals can look forward to living out their lives to the fullest. How, then, can the relatively vigorous health, effective functioning, and productivity of the middle years be continued into the later years? How can disability and dependency be postponed until the last years of the extended life course?

During 1981, SBR efforts have begun to lay the knowledge base for a program of research on these questions, involving a range of developmental activities, and have set in motion plans for implementation in 1982-1985.

Knowledge Base for the New Focus

Over half of SBR projects relate to prevention or reversal of age-related deficits. Recent research findings are beginning to suggest how the productive middle years might be extended; how many disabilities of old age might be prevented or postponed; and how the costs of health care and dependency might be contained.

Some SBR findings. For example, we now know that intellectual decline with aging (when it occurs) can often be slowed or reversed by relatively simple training interventions (Baltes and Willis); that older people can often learn to compensate for declines in reaction time, memory, and other age-related deficits (e.g., through mnemonic strategies, carefulness, and persistence - Perlmutter; Poon; Horn); that for the visual impairments suffered by many older people, particular styles and sizes of types can facilitate reading, and improved environmental design can offset inability to see large objects in low contrast (Vanderplas, Sekuler); that for the serious malnutrition of many older people, food can be adapted to the age-related changes in taste and smell that influence eating behaviors (Schiffman).

Some related findings. Widespread research reports are adding support to such recent SBR findings by showing, for example, that health can be promoted through changes in smoking, diet, and exercise across the life span; that complexity and challenge in a job has a continuing influence throughout life on people's psychological functioning and intellectual flexibility; that illness can be alleviated through social supports and improved coping behaviors; that many serious disabilities (even when experienced in nursing homes) can be reduced by regimens that reward activity and independence.

Some ongoing SBR projects. In addition to such findings already in hand, many SBR grantees are seeking to understand how to prevent, postpone, or reverse current disabilities of old age. Current projects address not only the well-known risk factors for cardiovascular disease, but also such topics as:

- Stress and coping - including studies of potentially stressful experiences such as bereavement (Vinick; Thompson; Dimond; Faletti; Helsing); retirement (Bosse; Friedman; Rosen) life events associated with aging (Lazarus; Lieberman; Wan; Thomas); the role of social support and social integration in protecting against the disease consequences of stress (Kahn; Jackson; Levine; Streib; Converse; Griffith; Lieberman; Lazarus; Thomas; Cohen; Faletti); coping strategies and the amelioration of stress through coping (Lieberman; Lazarus; Cohen; Faletti; Colby; Trimble; Cain).

- Accident prevention - including studies of aging and reaction time under pressure (Baron); selective attention in visual search (Nebes); cognitive and sensory deficits (Perlmutter); visual perception (Sekuler); and the relationship between sleep and daytime alertness (Dement).

Developmental Activities for the New Focus

Specification and extension of this knowledge base ties directly into SBR's concern with the interaction between psychosocial and biomedical aging processes, and several staff activities during 1981 are directly relevant. As a member of the Institute of Medicine, Dr. Riley has participated in various ways in the IOM programs, including the report of the Committee on Stress, and the implementation (with assistance from Dr. Abeles) of the findings from the six conferences on Health and Behavior: A Research Agenda sponsored by NIH/ADAMHA, a project for which Dr. Riley is cochair for NIH. Members of the SBR staff have worked closely with the developing programs in Behavioral Medicine at NHLBI and NCI; have attended meetings of the Academy for Behavioral Medicine Research (Riley); have organized sessions for scientific meetings on "The Effect of the Economy on Mental and Physical Health" (Suzman, for the American Sociological Association), "Gerontological Health Maintenance" (Abeles, American Public Health Association), "Psychosocial Factors in Health" (Abeles and Riley, International Congress of Gerontology), "Aging from Birth to Death: Biosocial Perspectives" (Riley and Abeles, American Association for the Advancement of Science); have published a chapter entitled "Beyond Ageism: Postponing the Onset of Disability" (Riley and Bond); have proposed an agenda item for the 1982 World Assembly on Aging entitled, "Maintenance of Health and Productivity in the Middle and Later Years."

In addition to these and other staff activities, efforts are underway through a Sources Sought announcement to re-establish a special Committee on Aging to replace the former Committee on Aging of the National Academy of Sciences (NAS). Originally designed as a standing committee of the NAS, the continuity of NAS funding was unfortunately disrupted by technicalities in the contract procedure. A Committee of this sort is essential as a cost-effective means of assisting in conceptualizing and implementing the goals of SBR's new program emphasis, and in pressing into the more stubborn and obtruse problems encountered. A neutral, unbiased organization is needed, that can draw upon the broadest national and international sources of information and recommendations regarding research issues and needs, and that carries the authority necessary to provide overview and advice.

Implementation of the New Focus

The specific initiatives so far planned to implement this new focus on Health and Effective Functioning in the Middle and Later Years are itemized under Future Program Directions. Other closely related initiatives are currently being planned in cooperation with BRCM.

Cooperative Relationships with BRCM

With the apppointment of the new Associate Director, Dr. Edward Schneider, and the reorganization of the program in Biomedical Research and Clinical Medicine, an important working relationship has been forged. The cooperation extends from administrative arrangements to several substantive areas of common concern.

Administrative Cooperation

Already in place, for example, are procedures for assignment of grant applications and for negotiations of priority ordering of applications to be recommended for high program relevance (HPR). Depending on their primary focus, new applications referred by the Office of Planning and Extramural Affairs are often by common consent transferred from one program to the other, or shared between the two programs via primary and secondary assignments. Potential disputes over HPRs are avoided through compromises agreed to in continuing discussion of the merits of each particular application.

Areas of Common Concern

Illustrative of current SBR grants that relate to BRCM concerns are studies in the following areas:

Nutrition. The SBR program includes studies of changing taste and olfactory quality with age (Schiffman); the biobehavioral effects of undernutrition and aging (Campbell); the effects of living arrangements on nutrition (Davis); dietary habits, stress, and social factors (Rodin); and migration, obesity, and nutrition (Pawson).

Exercise. Two longitudinal studies include some assessment of the effects of exercise on morbidity and mortality (Thomas; Sears).

Menopause. A set of studies deal with menopause in healthy women, examining: the correlation between menopausal symptoms and labor force participation, estrogen use, and the role of social supports (McKinlay); the natural history of menopause in peasant societies that neither use hormone therapy nor emphasize youthfulness (Haug); menstrual status as a baseline marker for aging in respect to a full hormone profile, the sex steroids and life health histories, assays of nutritional well-being, psychosocial adaptations, physical health, and physical fitness (Notelovitz).

Hypertension. SBR projects deal with the effect of hypertension on cognitive functioning, information processing, and biofeedback control of EEG (Elias; Woodruff); and several with taste changes which might be related to dietary changes, especially salt intake, affecting blood pressure (Schiffman; Stevens).

Biomedical Research and Clinical Medicine Program

Program Description BRCM-1

Program Highlights BRCM-1

BIOMEDICAL RESEARCH AND CLINICAL MEDICINE PROGRAM

PROGRAM DESCRIPTION

The Biomedical Research and Clinical Medicine Program (BRCM) supports research and research training for geriatric medicine, physiology of aging, and the molecular and cellular basis of aging processes. The Program was reorganized in 1981. It is now comprised of the Geriatrics Branch, the Physiology of Aging Branch, and the Molecular and Cell Biology Branch. The personnel associated with each branch and section are presented in Figure 1. The major objective of this organization is to encourage research on the basic aging processes and their impact on major organ systems and to facilitate the transfer of that knowledge to studies of geriatric problems.

The Office of Resource Development is responsible for administration of the aging animal colony and the cell line repository and the development of new models to support research on aging.

The Office of Program Development is responsible for the assignment of research grants within BRCM, development of grant funding recommendations, scientific analysis and preparation of the BRCM Sourcebook.

PROGRAM HIGHLIGHTS

The Animal Models Program supported through the contract mechanism has for several years provided standardized colonies of Sprague-Dawley and Fischer 344 rats. Because of decreased demand by investigators, the contract supporting the Sprague-Dawley strain was terminated in January 1981. The Fischer 344 will be continued since it is considered to be the best rat model currently available for aging research.

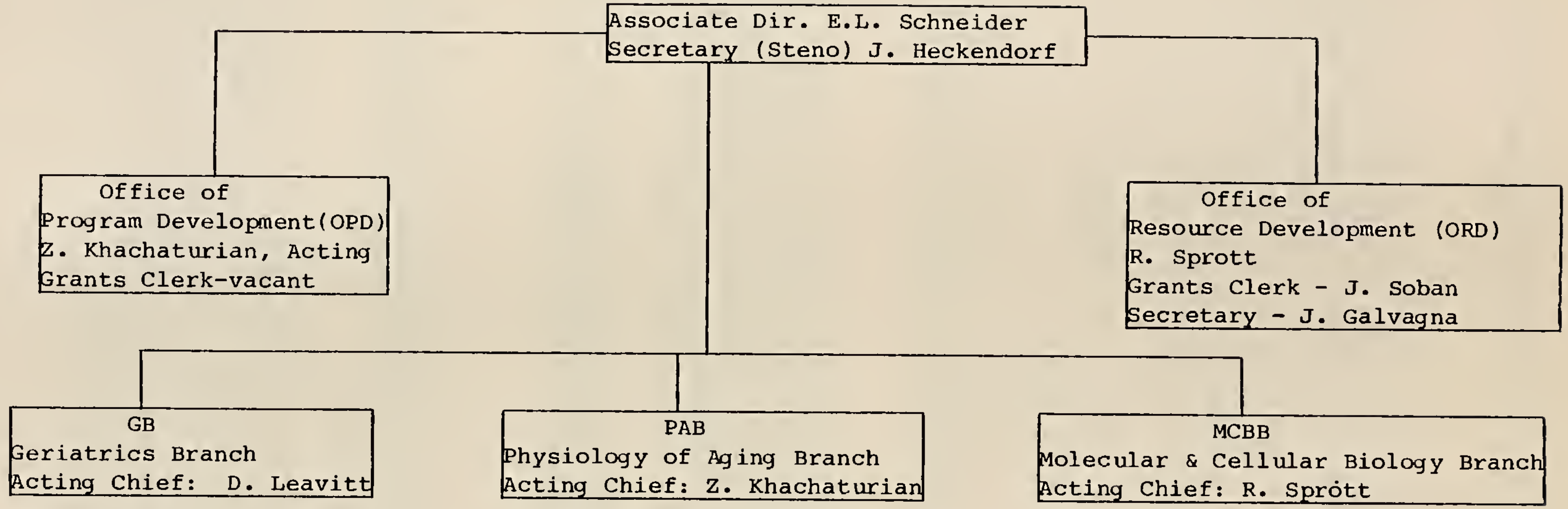
Five inbred mouse strains, three hybrid mouse stocks, three cogenic mouse strains and one outbred mouse stock are also maintained by NIA. The BALB/$_c$, C57BL/6, and the CB6F1 are in greatest demand.

The Geriatrics Branch

It has been estimated that by the end of this century over 25 million Americans will have attained the age of 65, many of whom will have multiple and complex illnesses requiring significantly more medical care per individual than will younger persons.

In accord with the Research on Aging Act of 1974 (P.L. 93-296), the Geriatrics Branch, BRCM, was established not only to support biomedical research related to the aging process, but to conduct training and other activities which are necessary to fulfill that portion of the NIA mission which addresses "the diseases and other special problems and needs of the aged." Major program elements within the Geriatrics Branch include the Pharmacology Section, the Geriatrics Research Section, the Dermatology Section and the Geriatric Training Section.

BRCM
Organization



Associate Dir. E.L. Schneider
Secretary (Steno) J. Heckendorf

Office of
Program Development (OPD)
Z. Khachaturian, Acting
Grants Clerk-vacant

Office of
Resource Development (ORD)
R. Sprott
Grants Clerk - J. Soban
Secretary - J. Galvagna

GB
Geriatrics Branch
Acting Chief: D. Leavitt

PAB
Physiology of Aging Branch
Acting Chief: Z. Khachaturian

MCBB
Molecular & Cellular Biology Branch
Acting Chief: R. Sprott

Sections

(PHR) PHARMACOLOGY G. Steinberg
(DER) DERMATOLOGY E. Hadley
(GET) GERIATRIC TRAINING E. Hadley
(GER) GERIATRIC RESEARCH E. Hadley
HSA vacant

Additional Personnel

Secretary - C. Saunders
Secretary - A. Simpson
Grants Clerk - J. Badinelli
Clerk-Typist(SIS) - vacant

Sections

(NUT) NUTRITION E. McGuire, expert
(EXR) EXERCISE PHYSIOLOGY - vacant
(END) ENDOCRINOLOGY B. Wortman(NEI)
(IMM) IMMUNOLOGY N. Braveman
(SEN) NEUROSCIENCE Z. Khachaturian

Additional Personnel

Secretary - D. Strong
Secretary - E. Cleveland
Grants Clerk - L. Wardrop
Clerk-Typist(P/T) - vacant

Sections

(CBY) CELL BIOLOGY N. Das
(MBY) MOLECULAR BIOLOGY R. Sprott
(GEN) GENETICS R. Sprott
(THB) THEORETICAL BIOLOGY R. Sprott

Additional Personnel

Secretary - vacant
Secretary(P/T) - M. Brophy
Grants Clerk(P/T) - K. Sacks

Figure 1

Pharmacology Section

The Pharmacology Section is supporting research on the incidence of adverse drug reactions and aging. It has been estimated that in the over 80 group, the incidence of adverse reactions may often be three times greater than that in the general population. Further, the use of multiple drug regimens leads to the greater probability of adverse drug-drug interactions in this population, which is probably exacerbated by the increased sensitivity of the elderly to untoward drug reactions.

It is well established that the elderly have increased responsiveness to the central effects of the benzodiazapines. The cause for this increase is under active study and recent reports suggest that the mechanism may be complex and subtle. Findings indicate that the increased risk of adverse drug reactions in the elderly to amitriptyline is not primarily due to age-related differences in drug distribution and hence must result from a change in pharmacodynamics with increasing age. With increasing age, postmaturity decreases have been observed in glutathione levels, microsomal mixed function monooxygenase activities and glutathione metabolising enzymes. It has been suggested that these changes may contribute to a decreased rate of metabolism and increased susceptibility to drugs, foreign chemicals and disease processes which occur with advanced age.

Dermatology Section

The Dermatology Section seeks to:

- establish normal values for dermatologic parameters in the elderly to facilitate evaluation of pathologic conditions;
- improve epidemiologic data on prevalence, incidence, risk factors, and complications of decubitus ulcers;
- increase understanding of cellular, tissue, and systemic changes which increase susceptibility to decubitus ulcers and impair wound healing;
- improve methods of preventing or curing decubitus ulcers and of accelerating wound healing in the elderly.

The bulk of activity in the dermatology program to this point has concentrated on descriptive studies of age-related changes in skin and connective tissue. These studies have demonstrated a diminished and delayed response to skin irritants in the elderly. This observation may have important implications for the diagnosis of inflammatory disease in old age.

The biochemical and cellular basis of wound healing in the aged rat is being explored and a minimally invasive system has been developed to examine the phenomenon in humans.

Geriatrics Research Section

The Geriatrics Research Section is supporting studies of disorders which are concentrated predominantly among older people (e.g., osteoporosis, benign prostatic hypertrophy, urinary incontinence) or which are associated with increased morbidity and mortality in the elderly (fall injuries, pneumonia). The medical management of these problems is complicated by underlying physiologic changes with age and the frequent presence of multiple disorders, often necessitating altered therapeutic strategies.

Program objectives are to:

- improve epidemiologic data on prevalence, incidence, associated risk factors, and complications of the major sources of morbidity among the elderly, particularly dementia, incontinence, hypo- and hyperthermia, pneumonia, osteoporosis, osteoarthritis, and fall injuries;
- determine normal age-related changes in those physiologic functions involved in the development or prevention of major causes of morbidity and mortality in the elderly. Determination of normal values for these functions in the elderly is essential for the evaluation of etiologic or predisposing factors for disorders of old age. Factors regulating cognition, memory, temperature regulation, sleep, micturition, resistance to infection, and bone metabolism are of particular importance.
- improve understanding of the etiology and pathophysiology of the above disorders.
- Evaluate effectiveness of present and new techniques for the diagnosis, prevention, treatment or cure of these disorders.

A large project to obtain a wide range of clinical pathologic data from autopsy and surgical specimens have been initiated. These studies will be valuable in establishing data on normal and pathologic changes with age in man. Another project to study changes with age in mechanical and ultrasonic properties of human bone has recently begun, and should yield important data in regard to osteoporosis and risk of fracture. Studies on impairments with age in response to cold exposure are in progress.

An epidemiologic study of international differences in mortality rates is adding to knowledge of genetic and environmental risk factors in major disease. Two epidemiologic studies are examining the role of physical activity, endocrine, and dietary factors in the development and prevention of osteoporosis, and elucidating further details on the relationship of osteoporosis to other risk factors for fractures. Clinical studies on response to cold exposure have shown an impairment in vasoconstriction with age that may contribute to the increased incidence of hypothermia in the elderly. Studies using an animal model of osteoarthritis have increased knowledge of cartilage changes with age that predispose to this condition. Clinical studies to elucidate neural and vascular problems associated with impotence in elderly men are in progress. Studies on defects in extraneural tissue associated with Alzheimer's disease should clarify pathophysiology of this disorder and faciliate antemortem studies.

Geriatric Training Section

The Geriatric Training Section has developed the following program objectives:

- develop improved curriculum in geriatric medicine and dentistry;
- increase research training opportunities in geriatric medicine and dentistry;
- develop faculty for the teaching of geriatric medicine and dentistry;
- facilitate interchange of educational ideas and methods among researchers.

Curriculum development has been a major focus of the awardees of the Geriatric Medicine Academic Award. Assessment measures undertaken by several awardees showed that, despite limited preexisting clinical geriatric curricula there was strong positive student interest in geriatric medicine.

Instructional activities increased significantly. Material on geriatrics was integrated into established courses and new electives were developed. Preclinical and clinical topics included were: physiology of aging, human growth, maturation and senescence, pharmacokinetics and pharmacodynamics of aging, urinary incontinence, and externships in geriatric medicine.

Geriatric research activity by students and faculty was encouraged and developed by the awardees. One principal investigator has designed a new assessment instrument for a prospective study of nursing home patients changes in their social and physical conditions. A student fellow initiated a research proposal which will establish norms for protein-caloric intake in an elderly population. Research on endocrine factors which affect sexuality in aging males is being continued by a principal investigator and another faculty member.

Participation in mandatory rotation in geriatric medicine for medical interns and residents, resource material development, annual conference establishment of fellowships and journal clubs were among other significant activities of the awardees.

The Geriatric Dentistry Academic Award program inaugurated last year is at an earlier phase of development than the medicine program. However, curriculum and research activities by the four awardees are developing in a parallel manner.

Molecular and Cell Biology Branch

To increase knowledge related to the basic aging processes, the development of age-related diseases, and the development of rational approaches to disease prevention and health maintenance, it is essential to understand aging at the molecular and cellular levels. This branch supports research on the mechanisms of aging through program activity in molecular biology, cell biology, genetics and theoretical biology.

The Molecular Biology Section currently supports research on intracellular information transfer, membrane structure, and extracellular structural components such as collagen, elastin, and glycosaminoglycans and changes in these components as a function of age. Using recombinant DNA techniques, several investigators are studying age-dependent changes in methylated DNA and its relationship to the regulation of transcription, changes in mitochondrial DNA and in mitochondrial lipid composition with age and their effects on cellular respiration. Others are studying age-related changes in the composition and conformation of collagen, dentin and ocular lens protein. These studies have provided evidence that post-translational changes of these cellular and extracellular constituents constitute a significant manifestation of the aging processes.

Cell Biology Section

This section supports research to understand the basic mechanisms and the consequences of cellular aging. Use of *in vitro* and *in vivo* cell systems, tissues and cell transplantation and chimeric and genetic mosaic animals are encouraged to gain knowledge on age-related alterations in patterns of cell growth, proliferation, development and differentiation.

In support of research in cell biology of aging, NIA maintains through a contract a cell-line repository at the Institute for Medical Research (IMR), Camden, New Jersey. This repository acquires, develops, characterizes, stores and supplies cultures for gerontological research. The repository contains cell-lines which include human diploid fetal lung fibroblasts (IMR-90/91), skin fibroblasts from individuals of different ages and fibroblasts from individuals of different ages and fibroblasts from individuals exhibiting features of premature aging, such as progeria and Werner's syndrome. In addition, the repository also maintains several epithelial cell lines. With a view toward providing quality control in cell culture technologies, as applied to gerontological research, this section maintained, through contract, the Mycoplasma Contamination Testing Service at IMR. This contract expired on March 31, 1981. It has provided excellent educational as well as mycoplasma testing services to NIA grantees during the three-year period of its existence.

One of the cell culture systems widely used in research on cell biology of aging is the normal diploid human fibroblast-like cells (HDF) of lung and dermal origin. HDF have limited proliferative capacity of "life span" in culture. The population doublings achievable by HDF is inversely proportional to donor age. Questions have been raised in recent years on the validity of HDF as a model system for cellular aging research. It has been argued that the loss of *in vitro* proliferative capacity is an indication of cell differentation rather than aging. Studies show that cells of all vertebrates do not show the Hayflick phenomenon or *in vitro* programmed senescence. The myogenic and fibroblast-like cells of lizard *Anolis* do not senesce in culture. These cells do not exhibit any signs of transformation in culture.

Techniques are now being developed to maintain HDF in serum-free media. Recent developments in cell-culture techniques have made is possible to culture various tissue and organ specific cells suitable for comparative studies of cellular aging. Recognizing that phenotypic, and perhaps genotypic, differences exist between cells _in vitro_ and _in vivo_, many differentiated cells do express known functional traits when maintained in culture. The use of differentiated cells in cultures (e.g., cell obtained from vascular and nervous tissues, gastrointestinal and urinary tracts, endocrine and exocrine organs, retina, lung, bone, muscle and skin) provides an excellent opportunity to study age-associated alterations in differentiated functions that are expressed by cells _in vitro_. Such studies could lead to an understanding of: mechanisms of age-associated functional decline in various tissues and organs and susceptibility to age-related diseases such as diabetes mellitus, osteoporosis, osteoarthritis, cancer, hypertension, and senile dementia of Alzheimer's type. Studies by NIA grantees show that bovine pulmonary artery endothelial cells have limited proliferative capacity in culture. These cells exhibit a considerable decrease in the activity of the angiotensin converting enzyme as they "age" in culture. This enzyme activates angiotensin I by converting it to angiotensin II and inactivates bradykinin. Since angiotensin II is a potent vasopressor and bradykinin is a vasodepressor molecule, this enzyme is of pivotal importance in blood pressure homeostasis.

Human epidermal keratinocytes can now be cultured in a serum-free medium. These developments now make it possible to obtain relatively pure populations of keratinocytes, to store these cells for use in a series of experiments to cause the cells to differentiate at will, and to separate the presumptive differentiated cells from the proliferating cells.

Somatic cell hybridization techniques have been employed to gain knowledge on genetic regulation of senescence and regulatory processes in cell proliferation and cell function. Studies in which hybrids were made by fusing individual cells and carefully noting the nucleocytoplasmic relationship, showed that both the nucleus and the cytoplasm control the characteristic life span of the cell. These studies further indicate that finite life span is dominant in most of the hybrids obtained by transferring SV-40 transformed HDF nuclei into the phase II normal HDF. The loss of replicative ability is perhaps due to accumulation of cell division inhibitory factors in these hybrids. Such an inhibitor (or activator) has yet to be isolated from these cells.

The somatic cell hybridization technique has been applied for chromosomal localization of genes. Studies with human-mouse and human-hamster cell hybrids have shown that the human chromosome number 21 contains several genes involved in purine biosynthesis. The gene for the soluble form of superoxide dismutase (which protects cells from free radicals) and the interferon sensitivity gene are also present in this chromosome. This chromosome in triplicate form causes Down's syndrome. Several studies have shown that individuals with Down's syndrome exhibit many features of premature aging, including premature brain changes of senile dementia.

Changes in the activity and structure (and/or conformation) of several membrane-bound enzymes have been noted during cellular aging. The composition of the extracellular matrix which plays an important role in the cell structure and function also seems to alter as cells age in culture. In addition, studies of several workers suggest age-associated alterations in the affinity of cell receptors to growth factors, hormones and neuroeffectors.

One of the cytoplasmic structures that increases in size and number as cells age is the lysosome. Lysosomes are membrane-bound structures which contain a variety of hydrolytic enzymes capable of digesting all major cell constituents. Several studies have shown that nondividing cells, such as neurons accumulate the so-called age pigment, the lipofuscin. Lysosomes are the sites of accumulation of this pigment.

Studies are now being conducted on the age-associated changes in the structure and function of the cytoskelatal system of cells as they age in culture.

The cell nucleus which contains most of the genetic information of the cell has received considerable attention of cellular gerontologists. Studies on the structure and function of the nucleus related to aging include chromatin composition and nucleosome organization, chromosomal abnormalities, pattern of DNA replication and repair, transcription and and ribosome production. Experiments are in progress to determine the effect of controlled mutagenesis on the _in vitro_ life span of diploid fibroblasts.

Reports are available suggesting a selective and substantial loss of hybridizable ribosomal RNA genes during aging in mammalian cells. The rate of this loss seems to be slower in long-lived (e.g., humans) than in short-lived (e.g., beagle dogs) animals. A selective loss of ribosomal RNA gene, if it does occur, could impair the functional capacity of the postmitotic cells of mammals.

Genetics Section

Research supported by the Genetics Section has encouraged use of the fruitfly _Drosophila_, the laboratory nematode _Caenorhabditis elegans_, the slime mold, _Dictyostelium_, the colonial flagelate _Volvox_, and protozoa. The expansion of Genetics Section research support will focus on human genetics and the mouse as a genetic model. Commonly, these organisms are the subject of a broad range of highly competitive research, of which gerontology is but a small part. As most such research is on developmental biology, these fields focusing on specific laboratory organisms are excellent for expansion to encompass the interests of the NIA.

The first of several possible genetics colonies of experimental animals recommended in _Our Future Selves_ was established by the NIA in September 1979. This resource, the _Caenorhabditis_ Genetics Center (CGC) supports the biological community at large through services as mutant acquisition, storage, characterization and distribution. The CGC is in its second year of operation, and is recognized, in addition to be a mutant source, the national focal point for nomenclature, the genetic map and world literature reference file.

The nematode plays a significant role in aging research. One investigator recently demonstrated nutritional arrest of developmental and aging processes in Caenorhabditis. These animals, when grown on either bacteria or a chemically defined medium mature in 2 1/2 to 3 days, reproduce for 3 or 4 days, then senesce and die in 20 to 30 days. However, eggs from a bacterially grown culture, when allowed to hatch and grow in the chemical medium result in a population of larvae remains arrested as small juveniles for 3 weeks or more. When these juveniles are returned to feeding on bacteria they resume their development, mature in 3 days, reproduce for 3 days, the commence the normal postreproductive aging process. Thus, Caenorhabditis that have been maintained as acting juveniles in chemical medium 1.5 days and then transferred to bacteria are vigorous, healthy, young-looking adults on day 18, while worms from the same population maintained on bacteria from birth are senescent or dead on day 18. The biochemical and genetic bases for this and similar phenomena are being investigated.

Another investigator, working with another nematode, Turbatrix, has seriously challenged the error theory of aging. He has shown that an altered enzyme (enolase) in aged Turbatrix results from changes in enzyme conformation and not because of errors in amino acid sequence resulting from faulty genetic transcriptional or translational processes.

Several studies are being conducted by NIA grantees to determine effects of nutrition and gene mutation on longevity in the fruitfly Drosophila. Longevity of various species of Drosophila is decreased (by 50 percent) when larvae are well-fed with yeast and kept in less crowded conditions.

The effects of cell death on developmental patterns in Drosophila has been studied to understand cell growth and developmental processes. Age-specific differences were observed between 1, 15 and 30-day old adults with respect to isocitrate dehydrogenase (ICDH) and G6PD/6PGD (glucose 6-P-dehydrogenase/6-phosphogloconate dehydrogenase) activity in the gut.

Studies with flour beetles have shown that x-irradiation increases the longevity of these organisms. This increase in the longevity is not related to decreased sexual or reproductive activity. It has been suggested that the increased longevity of irradiated insects stems from induction of DNA-repair capacity which would otherwise decline with age in different post-mitotic tissues.

Studies by an NIA grantee on the age-associated cellular alterations in the housefly show that the average maximum life span decreases under conditions of higher physical activity.

Theoretical Biology Section

The techniques and concepts of theoretical biology offer an alternative approach to developing and testing hypotheses. Extramural research involving mathematical and computer simulation and modeling of age-related phenomena and mechanisms are supported in this section.

Simulation studies are encouraged both as data-driven and as theoretical models. Data-driven studies are those in which the modeling and computer simulation are dependent upon experimental data for development.

The simulation models become valuable when sufficiently sophisticated to predict functions describable as an experimentally testable hypotheses. Data-driven simulation research is encouraged at the molecular/biochemical, physiological, and cellular (cell-linkage) levels.

One investigator has initiated a quantitative kinetic (computer) modeling of carbohydrate metabolism in the slime mold Dictyostelium to understand critical variables involved in differentiation and aging of this relatively simple organism. Both the experimental and simulation analysis show that throughout differentiation glucose availability is rate-limiting for the end product, trehalose, synthesis. A preliminary transition model of the citric acid cycle in this organism has also been constructed.

Physiology of Aging Branch (PAB)

The Physiology of Aging Branch contains five sections; Neuroscience, Immunology, Nutrition, Endocrinology and Exercise/Physiology. As a unit, PAB, through its component sections, supports biomedical research on those age-related physiologic processes at the tissue, organ, organ system and organismal levels and stresses the incorporation of those findings into clinical studies. It encompasses basic research in humans and animal models.

Neuroscience Section

The Neuroscience of Aging Program (NAP) at NIA supports research which focuses on the relationship between aging and the nervous system (NS). The program axiomatically regards the aging NS as an entity which has distinctly altered physiological attributes: consequently, viewing the aging NS is a subject worthy of investigation as a separate area of neuroscience.

To achieve its programmatic objectives, NAP encourages research in the following areas of studies:

- basic and clinical studies on Alzheimer's disease (AD) and treatable brain disorders of the aged;
- learning and memory disorders of the aged;
- age-related changes or impairments in sensory functions, e.g., auditory (presbycusis), visual, olfactory and gustatory; and decrements in motor functions or fine motor coordination, such as hand-eye coordination;
- sleep disorders, insomnia and sleep apnea in the aged;
- studies of the function, structure, physiology, biochemistry, pharmacology and biophysics of the aging nervous system, with particular emphasis on those changes that take place during aging;

- studies on 1) ultrastructure, transport, and permeability at the blood-brain barrier; 2) experimental modification of barrier permeability; 3) regional cerebral metabolism and blood flow in relation to brain function; 4) neuropharmacology in relation to stress, hypertension, and synaptic transmission; 5) neurophysiology of nerve, muscle, and synapses; 6) factors regulating neuronal death, as well as neuron-neuron and neuron-effector organ interaction; 7) neuroendocrinology; and 8) quantitative relation of regional cerebral metabolism to aging and dementia.

Among the topics listed research related to AD is of particular interest to NAP. AD is a chronic, degenerative disease of the nervous system seen predominantly in the elderly. It is characterized by distinct neuropathologic alterations consisting of prominent senile plaque and neurofibrillary tangle production in the neurons of the frontal and temporal cortex and the hippocampus. Postmortem studies have shown a striking correlation between the degree of clinical dementia and the extent of neurofibrillary tangle formations in the hippocampus. Dementia affects five percent of the U.S. population over 65, or one million people. An additional ten percent of the older population shows varying degrees of cognitive dysfunction. Studies at autopsy indicate that at least 55 percent of persons dying with the diagnosis of dementia have AD. Conservatively calculated, AD probably ranks as the fourth or fifth leading cause of death in the United States.

Athough the etiology of AD currently is unknown, evidence of increased amounts of aluminum in the brains of individuals with this disorder has been reported. Through the use of scanning electron microscopy with electron probe analysis, one investigator is investigating the relationship of the accumulation of intra-neuronal concentrations of aluminum with the formation of neurofibrillary tangles. They report that tangle-bearing neurons of the hippocampus from cases of AD demonstrate the presence of aluminum in the nuclear region. Adjacent nontangled neurons from these cases are virtually free of detectable intranuclear aluminum concentrations. There is considerable evidence indicating that AD can be characterized as a primary degenerative condition of cholinergic neurons in hippocampus and cortex. Furthermore, substantial data exists that drugs which increase central cholinergic activity can enhance the ability of normal people to learn new information.

In a related series of studies conducted on the brains of AD patients at autopsy another investigator has made several significant observations that the brains of AD patients have reduced levels of somatostatin-like immunoreactivity. The reductions in some brain regions correlate with reductions in choline acetyltransferase activity (CAT). Animal studies indicate that certain specific classes of dopamine receptors as well as histamine and serotonin receptors are lost with age.

Immunology Section

It has been established that immune potential declines with advancing age in all mammalian species tested. In addition, autoimmunity and immunodeficiences become more frequent in aged individuals. Substantial evidence for involvement of the immune system in aging has come from observations such as 1) involution of the thymus - an organ known to be essential for generating normal immune responsiveness as the aging process occurs; 2) shortening of the lifespan and occurrence of aging-like features in certain autoimmune-susceptible strains of mice such as NZB/W; 3) occurrence of features of accelerated aging in certain human diseases in which immune dysfunction is apparent (i.e., Down's syndrome, Werner's syndrome and systemic lupus erythematosus); 4) the frequency of occurrence in advanced age of human and animal diseases which may involve a compromised immune response (e.g., cancer, emphysema, maturity onset diabetes, adult rheumatoid arthritis, amyloidosis). A major goal of research in immunology and aging is to elucidate the relations outlined above.

The Immunology Section supports clinical and basic studies on the effect of the environment (diet, stress, exercise) on immunologic function; basic age-related changes in the immune system, e.g., antigen processing, lymphocyte/macrophage function and regulatory mechanisms and; clinical studies in humans (Down's syndrome), and animal models (amyloidosis and autoimmune diseases).

Since the observation of Dr. McCay on the extension of longevity in diet-restricted mice, the effect of diet on the immune system has been studied more intensively. One investigator has shown that natural killer cell (NK) activity in short-lived and long-lived strains of mice is correlated with longevity. Additionally, reduced NK activity in old NZB mice (a short-lived strain) is probably associated with a lack of precursor cells.

Another investigator is studying the effects of *in vitro* thymosin treatment on the immunologic responsiveness of human peripheral blood lymphocytes from normal and autoimmune aging individuals. Preincubation of peripheral blood leucocytes (PBL) from rheumatoid arthritis (RA) patients with thymosin appeared to induce a suppressor cell population not observed in similarly treated PBL from normal individuals. These data suggested that in RA patients, there may be an excess of suppressor cells and an excess of thymosin inducible precursor suppressor cells in comparison to non-RA individuals. This finding is in contrast to systemic lupus erythematosis (SLE) patients who are deficient in suppressor cells but whose suppressor cell activity can be restored by preincubation with thymosin.

Another investigator is studying the expression of the B-cell repertoire in mice during the aging process. His experiments have shown that on a per cell basis, mouse B-cells from spleen and bone marrow respond to an antigenic stimulus to the same degree as B-cells from younger mice although immunization of these aged mice respond to a lower level than do their younger cohorts.

Another investigator is studying age-related quantitative and qualitative changes in T and B-cells in humans. His data indicate that a defect in T cells is present in aged humans and this defect results in a reduced proliferative capacity.

Endocrinology Section

Research supported by the Endocrinology Section has provided increased knowledge on basic biological processes essential to prevent disease, improve human health and the quality of medical practice. Normal growth, development and maturation require growth, adrenocortical, parathyroid and gonadal hormones, as well as insulin and glucagon. Mental status is dependent on endocrine function and hormonal interactions. In some of the late occurring diseases and disabilities, such as hypertension, cancer, atherosclerosis and benign prostatic hypertrophy seen in humans may be hormone related and certainly aged related. Clearly the health and well-being of all individuals at all ages is dependent on an intact, functioning endocrine system.

During aging, there are striking changes in endocrine function that result in total cessation of ovarian function which then leads to the menopause and its sequelae. Other changes in the endocrine and neuroendocrine functions, such as deficiency in the production of a number of pituitary hormones undoubtedly have a major impact in the response of every body organ.

Molecular mechanisms casually related to senescence-associated development of diminished function and/or dysfunction remain largely undefined. The development of rational therapies for the management of aging-associated dysfunction may ultimately improve the quality of life or lead to the prolongation of useful life.

One project seeks to identify the major physiological causes underlying the decline in reproductive performance in aged male mice. Attention has been focused on defining functional alterations in the brain-pituitary-testis system during aging.

Clinical observations and experimental results suggest that a delay of ovulation is important in the production of developmental anomalies. The incidence of birth defects is greatest during the very early and late periods of reproductive life. There are times when the follicular phase of the menstrual cycle is often prolonged, which could result in intrafollicular aging of the oocyte.

The regulation of plasma calcium concentration is intimately related to the regulation of bone metabolism. The cause of the deleterious loss of bone that occurs with aging in humans remains unknown and consequently, a rational therapy for the disease is unclear. It is hoped that studies will clarify the changes which occur with aging in the actions of vitamin D, the calcium regulating hormones, calcitonin,parathyroid and growth hormone, and the interrelationships of the effects of these hormones on bone.

Studies of benign prostatic hypertrophy have established that dihydrotestosterone, the active intracellular androgen in the prostate of all species, accumulates in excessive amounts in the hyperplastic prostate; that prostatic hyperplasia does not develop in the castrated male but does occur under circumstances in which estrogen formation in the aging male is increased; and that dihydrotestosterone accumulation is greater in the periurethral region of the human prostate where prostatic hyperplasia commences than in the outer portions of the gland. These findings suggest that antiestrogens or antiendrogens could prevent the onset or induce regression this condition.

A study of adult-onset diabetes concerns the relative contribution by beta cells and by other cell types within isolated pancreatic islets of Langerhans to the previously reported inability of isolated islets from aged Sprague-Dawley rats to secrete insulin in response to glucose. Availability and/or action of somatostatin, glucagon and other islet secretory products are being studied with respect to glucose-stimulated secretion of insulin. Progress to date suggests an altered insulin secretory response within the beta cell, as well as a possible regulatory role for somatostatin. The deficiency in insulin production concerns the mobilization of stored hormone rather than the biosynthesis of insulin. Mobilization of stored insulin by isolated islets in response to glucose is altered during aging, whereas responsiveness to at least one other secretagogue, leucine, remains intact. The precise nature of this specificity will be determined by ascertaining effects of rat donor age on the integrity of insulin secretory response by isolated islets to a spectrum of secretagogues, including other carbohydrates, amino acids and glucagon.

Another study indicates that the average beta cell from aging rats secretes insulin far less efficiently than does the beta cell from young rats. Attempts to define the mechanism responsible for the age-related defect in insulin secretion have excluded the possibility that a unique disruption of some subcellular organelle (e.g., microtubules) or some unique interference with the progression of proinsulin conversion to insulin is the cause of the problem. Glucose oxidation is not normal, and certain key glycolytic enzymes may not function adequately in islets of older animals. Whether the changes noted are responsible for the observed decline in beta cell function are not yet clear.

Nutrition Section

Research supported by the Nutrition Section covers a wide range of topics and a large variety of methodologies. At one end of the spectrum are quite basic studies such as those aimed at determining the biochemical functions of individual nutrients, especially those related to changes associated with aging. At the other end of the spectrum are studies at the level of the whole organism.

Nutrient requirements, with few exceptions, have not been well established yet for the elderly. Most recommendations for the elderly are based on extrapolations from what is known of the requirements for healthy young adults. A major goal of the Nutrition Section is to establish valid nutrient requirements for the elderly and to determine the degree to which specific requirements are altered by disease and drug regimens common among the elderly. Nutritional status, both of individuals and of populations, is another important area for research. Correlation of nutritional status with subsequent morbidity and mortality can provide useful information concerning the contribution of long-term nutritional status to the etiology and pathogenesis of diseases prevalent in the elderly.

It is not only important to understand the role of nutrition in disease processes commonly associated with aging, but it is at least equally important to understand the role of nutrition in the aging process itself. It has been known for many years that for rodents prolonged food restriction results in an increase in lifespan. However, the mechanism(s) responsible for the increase are not yet known. In addition, since most of this work has been done in rodents, it is important to determine the effects of food restriction on the quality as well as the quantity of life before such a manipulation can be contemplated for use with humans.

The questions being addressed in the studies involving humans include the role which zinc status may play in changes in taste acuity in the elderly, the changes which occur in glucose metabolism with increasing age, the changes which occur in protein metabolism in the elderly using albumin as the protein for study, and the prevalence of subclinical malnutrition in a healthy elderly population and the effects of such malnutrition on subsequent morbidity and mortality.

Several interesting nutritional questions are being addressed by investigators using various animal models of aging. These include studies of the mechanism(s) whereby food restriction increases rodent lifespan; development of a rat model which will allow the separation of aging effects from those due to obesity; and studies of such dietary variables as protein, calcium, vitamin E and essential fatty acids as possible modulators of various age-related processes.

Exercise/Physiology Section

The objective of the Exercise/Physiology Section is to generate, promote and support basic research into the age-associated functional changes in tissues, organs, organ systems and those integrative hemeostatic factors associated with their regulation and control.

One investigator is continuing studies on thermoregulation in elderly men and women during physical exercise (desert walks). The results indicate that at a work rate that required 40 percent of aerobic capacity, elderly men and women thermoregulate, equally well. Additional data on body temperature, electroytes in sweat, blood biochemistry and body fat during exercise are being analyzed.

Research Contracts - BRCM

Office of Research Development

Contract Number: N01-AG-0-2101

Contract Title: Maintenance of a Long-Term Aged Rat Colony

Contractor: Charles River Breeding Laboratories, Inc.

Actual and Projected Costs:

FY 1982 - 800,000

Progress Summary:

The objectives of this contract are to support the maintenance of a large colony of Fischer 344 rats and to distribute them on request to investigators supported by NIA. This colony has provided the primary support for aging research which uses the rat model of human aging. At the present time a large amount of data has been collected on this model and it serves as a very important standard by which to compare results obtained with other animals.

Proposed Course:

FY 1981 is the first year of a two year renewal of this contract. This contract and the entire aging animal models program is being evaluated at this time. A recommendation for continuation with some modifications is expected. A contract will be awarded on a competitive basis.

Contract Number: N01-AG-7-2128

Contract Title: Development of a Colony of Multigenotypic Aged Mouse Strains

Contractor: Charles River Breeding Laboratories

Actual and Projected Costs:

FY 1981:	$1,171,672
FY 1982:	$1,000,000
FY 1983:	to be determined
FY 1984:	to be determined

Progress Summary:

The objectives of this contract are to develop, define, maintain and distribute commonly used strains of laboratory mice to serve as model systems for aging research in the disciplines of immunology, neurobiology, pharmacology, nutrition, endocrinology, genetics and behavior. This colony was developed in response to investigators' requests for a number of different genotypes. The availability of the new strains was announced in NIH Guide for Grants and Contracts, Vol. 10, No. 6, 4/17/81. Characterization of the strains is complete for half the strains and will be published in early FY 1982.

Proposed Course:

The developmental contract which was to expire in October 1980 progressed well. The contract has been extended to allow for the recompetition process to be completed. No sources other than the present contractor were able to meet all contract requirements and it was reawarded to Charles River through FY 1984. Characterization of all strains is expected to be completed and the results published during FY 1983.

Contract Number: N01-AG-0-2100

Contract Title: Selection, Production, Characterization and Distribution of Genetically Marked Cells for Aging Research

Contractor: Institute of Medical Research,
Camden, New Jersey

Actual and Projected Costs:

FY 1981: 228,875
FY 1982: 240,941
FY 1983: 257,878
FY 1984: 275,895

Progress Summary:

The contractor will provide leadership in the acquisition, banking, and distribution of differentiated cell lines for use in aging research. Furthermore, the contractor will contribute to the development of the field of cellular aging theory, concepts, and techniques through consultation and workshops in the area of cytogenetics in particular somatic cell genetics, and aging.

This Repository has established approximately 500 cell lines which induce the female and male fetal lung fibroblasts, fibroblasts from skin biopsies of normal humans of various ages, of individuals who exhibit futures resembling premature aging, Alzheimers disease and differentiated cells such as endothelium, smooth muscle, renal epithelium, and endometrium.

During the past year a total of 414 cultures were shipped to investigators in the U.S. and foreign countries. The contractor provides a limited karyology service to NIH grantees and screens all cells for *Mycoplasma*, bacteria, yeast and mold contamination.

Proposed Course

The cell models program is currently under evaluation. This will be completed in FY 1982 and recommendations incorporated when appropriate but certainly when the contract is renegotiated.

Contract Number: N01-AG-9-2113

Contract Title: Caenorhabditis Genetics Center

Contractor: Curators of the University of Missouri-Columbia, Columbia, Missouri

Actual and Projected Costs

FY 1981:	38,216
FY 1982:	41,130
FY 1983:	44,539

Progress Summary:

The objectives of this contract are to acquire, maintain, and distribute wild-type and mutant strain of *C. elegans* for biomedical research. The contractor will determine that these nematodes are free from contamination, and conform to visible phenotype discription, will conduct annual survival tests for new methods of storage, and will maintain and distribute the genetic map and nomenclature information. This colony is to be used primarily for investigators studying molecular genetics and developmental biology particularly as they impact on aging processes.

During the first four months of the second contract year, the contractor has received strains from other laboratories, maintained an active schedule of distributions, and developed and tested a computerized system for information retrieval.

Nearly 800 strains have been received and maintained. 146 total strains were distributed during year one of the contract.

Proposed Course:

The overall organization of the Center is appropriate and on schedule. The contractor has been providing services promptly as requested. The major goal of the next period (04) is to have the computor software functional for demonstration at the Cold Spring Harbor Meeting in May. In addition the Basic User's Manual, new version of genetic map, integrated updated list of references and list of available strains will be made available. This contract and colony is also being evaluated and recommendations will be incorporated when appropriate and when the contract is renegotiated.

Immunology Branch

Contract Number: N01-AG-9-2100

Contract Title: Longitudinal Study of Immune Function in Thymic Hormone-Treated Mice

Contractor: University of Alabama, Dr. Hiramoto

Actual and Projected Costs:

FY 1981:	111,037
FY 1982:	152,000

Progress Summary:

The objective of this contract is to determine the effect of thymic hormones on the development and aging of the immune system. Two studies were identified to evaluate the effect of four thymic hormone preparations in three strains of mice. In one study, a longitudinal one, mice would be periodically treated with thymic hormone throughout life and monitored every three months for immune function. In a second study, a cross sectional one, mice of various ages would be treated with the hormone preparations and periodically assayed thereafter for there effect on different immunologic functions and pathological conditions.

Results - As of the most recent progress report (January 1981) the following progress has been made: A/JN, CFW and C57BL/6 strains of mice have been evaluated longitudinally through 12, 14 and 16 months of age respectively and: 1 month old CRF, 1 month old C57BL/6 and 24 month old C57BL/6 mice were treated in the cross sectional study. The data from the cross-sectional are too meager to analyze. In the longitudinal study, each strain has been evaluated from four to six times. The mice have reached middle age and although some variations from the control groups has been observed, specific effects of the hormone treatment have not been observed in mortality, weight, hemotocrit, numbers of peripheral blood white cells, Coombs anti-red cell agglutinin titers, anti-red blood cells hemolysis and anti-nuclear antibody.

Proposed Course:

The workscope is being adhered to faithfully and the experiments are being performed on schedule. The contractor is conducting the study in a highly competent manner. The results are too limited to assess at this stage.

The contract will be modified for the FY 1982 year to include appropriate controls for the "nude" mouse studies to validate the data derived.

Epidemiology, Demography, and Biometry Program

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Overview of NIA's Epidemiology, Demography, and Biometry Program

We take pride in having significantly furthered our scientific mission in a year beset with administrative and personnel problems. The National Institute on Aging has received final authorization for the reorganization which now establishes Epidemiology, Demography, and Biometry as a Program. Thus, we are now able to divide our Program along the initially intended lines with three proposed sections, which will become branches as their size increases. These are: 1) Epidemiology, 2) Demography and Economics, and 3) Biometry. Healthy interaction among these disciplines remains the underlying and practical mechanism of operation. As in the past, our research is conducted primarily by EDBP staff supplemented and augmented by research contracts and interagency agreements, professional services contracts, and numerous working arrangements with other Government and nongovernment peers and agencies. Our budget for this year is approximately 4 million dollars.

We were fortunate that the drastic personnel changes which occurred during a very difficult transitional year in Government, resulted in even stronger and more promising scientific personnel. The professional staff consists of Jacob A. Brody, M.D., Epidemiologist, (Associate Director for EDBP, NIA), Joan Cornoni-Huntley, Ph.D., (Acting Deputy Associate Director for EDBP, Expert appointment which expires in April 1983), Douglas A. Parker, Ph.D., Sociologist, (IPA whose appointment will terminate in July 1981, now serving as Acting Head of our Social Epidemiology Section), Lon R. White, M.D., M.P.H., Medical Epidemiologist, who is serving as Acting Chief, Epidemiology Branch, and Dennis Cosmatos, M.S., Health Statistician, now assigned to the Biometry Branch. Within the past 3 months we have added Dwight B. Brock, Ph.D., Mathematical Statistician who is Acting Chief of the Biometry Branch; William S. Cartwright. Ph.D., Economist, who is Acting Chief, Demography and Economics Branch; and Daniel J. Foley, Health Statistician assigned to the Biometry Branch. During the year we lost the services of Yuko Palesch, M.S., Health Statistician, and have not been able to replace the head of our research demography group.

The entire EDBP is located in the Federal Building which has the advantage of allowing us to occupy contiguous space enhancing our professional interaction but, of course, the disadvantage of geographic separation from the remainder of NIA.

In the evolution of EDBP the planning and issuing of major contracts and interagency agreements for most of our larger commitments has been moving along in a roughly parallel fashion. Thus during the latter part of fiscal year 1980 our major awards were made (Population Studies, HANES, Macroeconomic Model, Systolic Hypertension). This year has been characterized by the implementation of the machinery to provide for proper funding, develop questionnaires, and submit concept and forms clearance through the PHS and OMB. I am proud to say that my group has responded and we are on or ahead of schedule in most of our major endeavors. In future years we will be receiving the data we are purchasing. Hopefully, the size and skill of our staff will increase to the level where we can fully exploit the richness within these studies.

The "Establishment of Populations for Epidemiologic Studies of the Aged" being conducted by Yale University, The University of Iowa, and The East Boston Neighborhood Health Center (Harvard), is at the stage where the common questionnaire for the identification, description, and surveillance of the 12 to 13 thousand individuals age 65 and over to be studied has been agreed upon. Each study group will administer this common questionnaire while also initiating individual questions and substudies relating to specific areas of interest. We expect the extensive effort the EDBP staff has invested in coordinating, guiding, and integrating these studies will provide us with a product combining medical, social, and behavioral aspects of health by using integrated methodologies in these three separate and distinct populations, the similarities and differences we encounter will substantially enhance the value of the research projects. Through followup of these populations over the next 4 years and through thoughtful and timely additions to the basic protocols, these studies will generate information of great use and interest to NIA and will provide a valuable resources for all Government agencies interested in research and care of the elderly.

Our efforts to conduct a follow up of the National HANES I to determine the health effects of nutrition is progressing. Unremitting pressure from the EDBP staff is maintained to assure that the study is properly integrated and implemented. During the year we have negotiated with NCI, NIAMDD, NHLBI, NIAAA, and NIMH for partial support of this costly endeavor and for the most part we have been successful. NCHS subcontracted for the field studies. In addition a mechanism for tracing and locating original HANES I participants is established. The final questionnaires are now ready for forms clearance.

Our joint study with NHLBI, the Systolic Hypertension in the Elderly Program (SHEP) is going through the lengthy process toward initiating a feasibility study to determine how best to proceed with a randomized drug trial for the treatment of isolated systolic hypertension of the elderly. The working protocol was agreed upon by the co-investigators and the two Insitutes and the specially constituted coordinating and oversight committee in mid-December in 1980. Many meetings preceded and have followed the acceptance of the protocol. Final details are being considered by this highly competent, gifted, and experienced group of investigators. During the year the utmost attention was devoted to quality control, compliance, quality of medical care, medication, and changing of dose and type of drugs, etc.

The EDBP senile dementia research effort has continued with major studies in place and new ones about to commence. Through our interagency agreement with NIMH which augments their catchment area in New Haven (Yale University) we are developing prevalence information on mental illness in the population 65 and over. Last year our report alluded to the various administrative difficulties the entire NIMH Epidemiologic Catchment Area program was encountering. Recent progress has been gratifying, however, and the sampling is near completion. At the May 1981 meeting of the American Psychiatric Association, (New Orleans) a symposium is being conducted by the Yale group which will include six papers. One entitled "Cognitive Loss and Disability in the Elderly" will evaluate the general information on the cognitive aspects contained in the basic questionnaire. Specific data relating to prevalence of senile dementia within the limitations of the survey instruments used should become available during FY82. A logical second step after establishing prevalence of senile dementia in a community is to study the natural history of the disease as it occurs in a noninstitutionalized population. We emphasize this population source because adequate studies of institutionalized dementia patients with senile dementia are being conducted. We issued a request for contract for this difficult study entitled "Senile Dementia: National History in a Noninstitutionalized Population" in FY80. During 1981 we have had two ad hoc peer reviews of the applications received. At present we are still negotiating with the several research groups which received the highest recommendations from the review committee. This study is important but difficult since it requires an epidemiologic approach to a population, a medical/clinical approach to the individual, and a cognitive/psychological approach to the long term followup of the individuals. Limited efforts are proceeding in studies of possible familial association of senile dementia and Down's syndrome. Thus far, the group involved in this work has experienced difficulties in establishing cases and kindreds. An excellent EDBP commissioned report was received which reviewed, evaluated, and compared several field instruments for the detection and classification of dementia in order to define which offered most utility in given situations. While no instrument considered is totally adequate or sufficient the study supported the utility of the Mental Status Questionnaire (MSQ) for large scale open population surveys.

Potential sources for further information on incidence and risk factor association for senile dementia exist in the analysis of the National HANES Followup referred to above. In our three populations under surveillance, prevalence and incidence of senile dementia will be detected; and to the extent possible, within time limitations and survey techniques, we will also explore the incidence and prevalence of depression among the elderly. Dementia and depression will also be investigated in two studies of the Framingham Population which will be discussed below.

Through a complicated funding mechanism, the NIA Demographic-Macroeconomic Model of the United States is under development with the cooperation of the President's Commission on Pension Policy. Scientific inquiry about the phenomena of population aging and its impact on the retirement income system is being conducted. This research is facilitated through the integration of eight models in our current system: Hudson-Jorgenson Macroeconomic Model; Anderson Labor Market Model; ICF Inc. Population Model; Private and Public Employees Models; Social Security Model; Supplemental Security Income Model; and Medicare Model. Documentation of these models and a research report on the well-being of the elderly under selected pension policies will be completed this fiscal year. As of May 1981 the EDBP with its new staff economist will have sole responsibility for the macroeconomic model which is already on the NIH computer. Future plans include enhancements to determine the demand and supply of health expenditures as well as the effect of demographic change on disaggregated consumption expenditures.

It is important to the medical profession and to patients and their families to have a better understanding of how people die. For example the percentage of people who die painlessly in their sleep versus the percent who die in pain is unknown. We know very little about who is aware that he is dying prior to his demise and which patients receive adequate versus inadequate medication for pain. Better understanding of the realities of the last days of life would benefit families as well as the medical profession and other social institutions from hospice to church. For several years we have been trying to evolve a cost-effective study to provide these essential data. In an open population approximately one person per thousand dies per year. Thus, in order to have a study population of 1,000 we would have to follow a population of 100,000 for a year. We have tried to find population surveys upon which we could graft certain questions, but thus far all attempts have been unsuccessful. Recently, we have attempted through contract mechanisms to develop a useful, if not less definitive retrospective study, which would rely on death certificates as the source of decedents. Two professional services contracts have provided information suggesting the need for this study and some of the possible approaches. Shifts and declines in our current staffing as well as competing demands for our funds, however, may force us to postpone implementation of this important research undertaking.

We continue our interest in alcohol problems and aging and are impressed that from 10 to 25 percent of all health contacts made by people over 65 are reported to have some alcohol related component. Dr. Brody serves as Vice-Chairman of the Blue Ribbon Study Commission on Alcoholism and Aging and presented the keynote address at the National Council on Aging's meeting this year. Our recommendations have included limited surveys and considerably more attention to the role of alcohol abuse in general medical problems of the elderly. In addition, we are utilizing data collected in a large survey of Detroit relating to occupational conditions, health problems, and alcohol use among employed men and women. Our survey extended into the "young aged population" between age 55 and 74 which comprised 180 or 14 percent of the sample. Relationships for both sexes at all ages concerning drinking and diminished cognitive functioning and also the effect of certain types of employment on drinking behavior are emerging.

Our studies of the effects of climate on age-specific mortality proceed slowly. We believe this study is important because of wide seasonal variations in mortality of the elderly. This relates to hypothermia as well as hyperthermia and perhaps many unrecognized climate and environment associated events. A major problem was overcome when the National Climatic Center provided us with 30 tapes containing data going back some 30 years. We previously had acquired U.S. mortality data since 1962. Our immediate problem is that the mortality data are coded by state and county while the meteorologic data are coded by longitude and latitude. We are now searching through other Government offices to determine if there is a program which converts longtiude/latitude data to counties. If not, we will have to develop such a mechanism to map the climatic data into the appropriate counties in the United States. We are also adding the User Prompted Graphic Data Evaluation (UPGRADE) System from the President's Council on Environmental Quality. This is a multi-faceted system with a wide array of variables by geographic locality so that in addition to mortality and temperature the effects of air quality or source of energy or water, etc. could be integrated. This in-house task is a long term investment which will have special meaning for our Institute since the majority of deaths occur in the elderly. Our Biometry Branch is expanding studies of cohort mortality. Previously we showed that death rates from cardiovascular disease for women have been declining since 1940 (and probably since 1920) while death rates for men were gradually rising and suddenly declined around 1965 regardless of the date of birth of the men. This suggests that an environmental or exogenous factor acting at that time was the principle cause of the declining cardiovascular disease mortality among males. The male slope, however, is still not as steep as the female slope and so the excess life expectancy among females over males continues to increase. Recently we have been studing the patterns of death among males and females by race from the five major causes of death for all ages. We are developing information which strongly suggests that the majority of deaths among the elderly is from cardiovascular disease and that the risk of death due to cardiovascular disease continues to increase exponentially with age. Deaths from cancer gradually level off with increasing age until they actually comprise a smaller percentage of all deaths (either by number or rate) than cardiovascular disease. The implications of this finding are important in terms of possible insights on physiologic aging, morbidity, and mortality. Studies of comparative mortality and their physiologic and biologic interpretations will occupy considerable time of the program particularly with heightened interaction between biometry and epidemiology.

EDBP continues its association with the Bureau of Census through an interagency agreement with Jacob Siegel's group. We are expecting an update in the Special Studies Series entitled "Social and Economic Characteristics of the Older Population: 1978."

In collaboration with the Social and Behavioral Research Program we continue to transfer money through an interagency agreement to AoA to support the National Aging Data Archive at the University of Michigan. The Michigan group now has their program developed with greater sophistication and access and have made special efforts to make their computing facilities and data archive, containing approximately 900 data sets, directly accessable to NIA.

Our attempts to develop studies of a series of drugs including estrogens, sleep medication, and digitalis have not progressed because of changes in personnel and lack of adequate sources for data. Through our epidemiologic population studies, the HANES Followup, and other investigations we are proposing in the District of Columbia, we will be studying estrogen related problems and perhaps in time the cardiac glycosides. At present we have a small professional services contract with the North Carolina Pharmaceutical Research Foundation, University of North Carolina at Chapel Hill where surveillance of drug use is underway.

A collaborative study with HCFA on nursing home populations was completed. The most significant activity was the adaptation of a method to determine the length of stay in nursing homes. As a follow up, EDBP will analyze the newly determined length of stay in episodes of care for the major chronic conditions affecting the elderly nursing home population. We are increasingly impressed by the fact that nursing homes must be characterized in terms of the number of years in which they have been in service. Over a period of 5 to 10 years the population of nursing homes becomes more and more unchanging as the sickest die and healthiest return home leaving the chronic slowly progressive patients with limited opportunities to find other placements occupying the overwhelming majority of the available beds.

We are looking for ways to utilize the population of the District of Columbia for studies of diseases with possibly important differences in incidence or outcome between blacks and whites. The District of Columbia offers a unique opportunity since it has perhaps the largest and most stable middle class black population of any major city in the United States. Thus, we can compare certain diseases between blacks and whites while controlling for potential biases caused by disparaties in socioeconomic positions. Our first studies will involve hip fractures which are reported to occur three to five times more commonly among white females than among black females and be relatively rare among older white or black males. The possible role of estrogens in osteoporosis will be one of the variables considered in this investigation.

In fiscal year 1979 we transferred funds to NHLBI to complete an analysis of a carefully constructed disability survey of the Framingham population. The analysis is now complete and we have outlined five initial publications to be developed from the data. EDBP personnel will collaborate on two of these publications concerning the relationship of cerebral vascular accidents to physical disability and to the relationship of heart disease risk factor status to physical disability among persons with no history of cardiovascular disease. In addition we will take leadership in designing a broader analysis of the meaning of disabilities and disability scores in terms of health and detectable illness. We will attempt to relate disability scores to the prior known health conditions which were routinely recorded in the various Framingham examination cycles. Since the disability study was done in the 13th and 14th cycle, and we are now up to the 16th cycle, we can also determine the predictive value of disability scores. Disability scores have usually been ascertained in surveys based in the subjective answer of the person being interviewed. To the best of our knowledge this will be a unique attempt to validate the physiological significance by comparison with concurrent, prior and subsequent examinations and determinations.

We are also developing a new protocol to study senile dementia (and other conditions of the elderly) in the next cycle of examinations in Framingham. Approximately 4 years ago very detailed psychological testing was conducted on 2,800 of the approximately 3,200 members of the Framingham study. At that time 148 were diagnosed as dementia patients. We intend to examine the entire surviving cohort and administer appropriate tests of psychological and cognitive functioning. We will attempt to determine the incidence of dementia among the 2,800 people previously examined and establish a baseline for the remainder of the population. In addition, we will be able to follow the development, natural history, hospitalization, etc. of those who apparently had senile dementia 4 years ago. We anticipate further utilization of this population to determine patterns of nursing home use in relation to dementia and, to the extent possible, other conditions such as incontinence, hip fracture, the broad spectrum of physical and mental disease, as well as social support and living arrangements.

Overview of Epidemiology Branch

The FY81 research efforts of the medical epidemiology section mostly involved activities related to our three "Establishment of Populations" studies. These efforts were focused on development of baseline questionnaires and end point definition instruments and on other methodology considerations. As a result, we are assured that the contractors will be able to provide us with the data we will need to carry out the studies we plan to do when the data are delivered to us during the course of and at the end of the contract interval. This effort accounted for the largest part of our research activities during FY81 and promises to be well worth the attention it received. We were similarly involved in development of the questionnaire and methods to be used in the HANES I followup study, also expected to yield a great deal of information for analysis late in FY83. Substantial effort has been made by the EDBP staff to insure that the information which will emanate from these four large studies will be complementary, will be collected and processed in such a way as to allow for comparisons to be made among the three populations studied, and will emphasize research areas of special interest to NIA (such as sleep disturbance, medication use patterns, dementia, determinants and indicators of aging, etc.). In anticipation of the data which will become available from the HANES I followup study, we have begun a series of studies on constipation, bowel movement frequency, and use of medications for bowel problems. Initial analysis of data from the original survey has shown that, with increasing age, there is an associated marked increase in the prevalence of perceived constipation and in the proportion of persons describing regular use of medication (presumably laxatives). Nonetheless, there was little age-related change in reported frequency of bowel movement. Current analyses are focused on patterns of illness, drug use, reported diet, etc., among groups of individuals who reported different bowel movement frequencies, controlled for the individual's perception of himself as constipated or not, and for patterns of medication use. Information derived from these studies will be of use in the development of hypotheses related to morbidity and mortality outcomes which will then be tested when the followup data set becomes available.

Our efforts related to senile dementia were several in FY81: a very useful review of methods and instruments for the identification of dementia was completed and received as a result of a professional services contract; a study (sponsored by NIMH and supplemented by NIA) designed to determine the prevalence of dementia in the aged population of New Haven got underway and is expected to yield that information in the upcoming fiscal year; a study (professional services contract with the University of Washington) aimed at examining the familial occurrence of senile dementia in association with Down's syndrome was begun; seven proposals for the study of the natural history of senile dementia were subjected to two reviews, as a result of which we expect the awarding of two contracts in the very near future; and the foundation was laid for a very promising study aimed at examining risk factors, differential diagnosis, incidence, and prevalence of dementia among participants in the Framingham Heart Study.

Activities in the area of prescription drug use by the elderly were reduced in FY81 because of the departure of the staff person (Palesch) who had been most directly involved. The specific aspects of this research program which we plan to continue involve the descriptive epidemiology of digitalis and of hypnotic drug use.

Another area of special interest to the medical epidemiology section deals with the possible relationships between: early life health, growth, nutrition, family structure, and environment; patterns of reproductive life history; and late life health and longevity. An underlying purpose is to examine the epidemiology of aging within a conceptual framework derived from experimental animal studies, evolutionary theory, developmental biology, and the entire field of chronobiology. These interests are evident in certain of the questions we hope to have included in the questionnaires to be used in the HANES I followup and in our "Establishment of Populations..." studies. In addition, a feasibility study was completed which involved 600 individuals who were weighed and measured as children in Hagerstown between 1933 and 1945. By review of available records it was determined that approximately 20 percent participated in a community survey in 1974 as part of which blood pressures were obtained. Extrapolating from the known 33 percent community participation rate in that survey, it is estimated that approximately 60 percent of the original cohort was still alive and residing in the Hagerstown area in 1974. Although the small number of individuals for whom data were available precludes any definitive statement, no clear relationships were identified between childhood relative weight and adult blood pressure. This study was supported by a professional services contract with The Johns Hopkins University School of Hygiene and Public Health and was carried out by Dr. G. Comstock and Dr. M. Farmer, an NIA epidemiology trainee studying at that same institution until June, 1981, when she will return to this office. We hope eventually to carry out a much larger study, either in this population or another, in which prospectively collected measurements of childhood height and weight (as well as recalled information on early life conditions and reproductive life events) can be related to adult habitus, survival, and health in later years.

The FY81 projects of the social epidemiology section can be grouped into three general categories: industrial gerontology, alcohol and aging, and death and dying. Industrial gerontology is defined here as the study of the employment and retirement problems of middle-aged and older workers. Alcohol and aging is defined as research on the determinants and consequences of alcohol use and abuse among older persons. Death and dying is conceived of as the study of the psychological, social and health experiences of those persons in the last days of life.

We anticipate a continuing need for studies in industrial gerontology, largely because of the population age structure changes. The long-term decline in both fertility and mortality rates should be accompanied by a similar "graying" of the labor force in the last part of this century (U.S. Department of Labor, 1979). In addition, the recent interim report of the President's Commission on Pension Policy (1980) recommends that, "Older workers should be encouraged to remain in the workforce to add to their retirement income, to increase productivity and to contribute to psychological well being" (p. 49). The report also recommends that disincentives to work such as the retirement or earnings test be eliminated. If these changes are implemented, there may be a sharp reversal of the trend for older workers to comprise a decreasing proportion of the labor force.

We are currently analyzing data from a survey of 1367 employed women and men in metropolitan Detroit for the purpose of developing and testing researchable hypotheses in industrial gerontology. Data collection for the Detroit study was initiated while Dr. Parker and Dr. Brody were affiliated with the National Institute on Alcohol Abuse and Alcoholism (NIAAA).

In response to a request from the NIAAA, Dr. Parker and Dr. Brody presented some prevalence estimates of and risk factors for alcohol problems at a conference on Alcoholism in the Workplace in May 1980. Other analyses of the Detroit data which are in progress or planned include the following: 1) an examination of the effects of occupational conditions, age and alcohol use on cognitive functioning, 2) a description of the job conditions and health practices of older workers in Detroit, 3) an assessment of age and class as predictors of self-esteem and personal mastery, and 4) an analysis of the distribution and determinants of psychological distress in different age groups of employed persons.

Drawing upon the research on men by Dr. Melvin Kohn and his associates at NIH, the Detroit study is designed to assess the perhaps different responses women make to conditions of work and family life. The Detroit study has extensive data on job conditions (e.g., supervisory relations, complexity of work, routinization, time pressures), psychological functioning (e.g., anxiety, depression, cognitive functioning, self-esteem, personal mastery, job stress, job satisfaction), and alcohol use and abuse (e.g., frequency of comsumption, quantity consumed per occasion, lifetime consumption, frequency of intoxication, and alcohol problems). The Detroit study also has data on household labor, financial conditions, and some life change events.

Perhaps the most immediate problem facing older Americans is declining real income because of fixed pensions or modest social security and other transfer payments which fail to keep pace with taxation and inflation, a problem particularly acute since late 1978. A study proposed by the social epidemiology unit would examine the impact of financial distress and other life changes on the health and health-related behavior of middle aged and older Americans in a followup of the sample studies by Dr. Loenard Pearlin in Chicago. The study would also examine the possible mediating effects of different kinds of social support and belief systems. In addition, the research would attempt to determine if the older aged as compared with the middle aged have different ways of coping with life changes.

There are two general sources of financial distress affecting health and health-related behavior that would be considered in this resarch. First, there is the "poverty" or "lower class" effect of simply not having enough income to purchase basic essentials--food, clothing and probably substandard housing which can increase the risk of health problems. And second, there is the "downward mobility" or "change" effects of a decrease in or reduction of financial resources which can be stressful and place persons at greater risk for health problems.

Previous research has found effects of economic changes on mental illness, on alcohol consumption, and on mortality. This research, however, has not focused on the elderly as a unique population. Furthermore, the research has not considered how the effects of economic instability change with age, how the possibly mediating effects of social support and belief systems change with age, and how the strategies people use to cope with life problems change with chronological age. Our proposed research would seek to fill in some of these gaps.

Our social epidemiology section continues to support the concept of an NIA-sponsored conference on clinical and epidemiology issues related to alcohol and aging. Particular attention would be given to the effects of employment and retirement on alcohol use and abuse among middle-aged and older workers. Dr. Parker and Dr. Brody have found certain aspects of employment--job complexity and job stress--are asociated with alcohol problems. Retirement with its attendant boredom, change of status and loss of income may also be related to alcohol problems. The conference was approved in concept in July 1979 but the implementation of the conference has been deferred until more detailed plans have been developed.

During FY 81 our social epidemiology section continued efforts to develop the first in a series of projected epidemiological studies of the last days of life. Because the great majority of deaths occur among the aged and the events and circumstances immediately preceding death are poorly understood, the National Institute on Aging is concerned with the development of systematic information on the last days of life from spouses and significant others of deceased persons and from attending physicians who had medical knowledge of the deceased. For the purpose of this study, the last days of life are defined as the last month of life. The objective of this initial study is primarily descriptive; we wish to develop data concerning how people die; at home or in a hospital and if in a hospital for how long prior to death, in pain or comfortably asleep or at rest, if in pain the amount, duration, and type of medication used; whether death was expected, medical and paramedical contacts and visits from friends, family members and others during the last days. Because previous research suggests that the circumstances of death are dependent upon sociodemographic variables such as race and income, this study should be conducted in locations with differences in ethnic background and variation in social class. A probability sample of death certificates will be drawn. From information provided in the death certificates, and from other information which can be obtained, e.g., from obituary columns, we will compile lists of potential respondents such as spouses who had personal knowledge of the deceased and attending physicians who had medical knowledge of the deceased. Using selection procedures developed in consultation with a sampling statistician, we will interview some of these potential respondents concerning the last days of life. The time period involved in this contract is estimated at 3 years, with 12 months for development of the sampling design and construction of the survey questionnaire, 6 months for OMB review and approval, 12 months for data collection, and 6 months for data processing and completion of the final report on methodological procedures used in the study.

A professional services contract with Dr. Dean Echenberg was initiated during the fall of 1980 in order to produce a questionnaire for pilot testing in studies of the Last Days of Life. We expect to receive Dr. Echenberg's final report by the end of May 1981.

OUR SOCIOLOGY SPECIALIST IS LEAVING THE PROGRAM. ALL ONGOING AND PROPOSED PROJECTS INVOLVING THE SOCIAL EPIDEMIOLOGY SECTION WILL BE SUSPENDED PENDING AVAILABILITY OF A PERSON WITH SIMILAR EXPERTISE AND INTERESTS.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 02010 03 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Followup of Health and Nutrition Examination Survey I (HANES I)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joan Cornoni-Huntley, Ph.D.	Epidemiologist	EDBP	NIA
OTHERS:	Jacob A. Brody, M.D.	Associate Director for EDBP	EDBP	NIA
	Dennis Cosmatos, M.S.	Statistician	EDBP	NIA

COOPERATING UNITS (if any)

National Center for Health Statistics, Division of Analysis; NCI, Environmental Epidemiology Branch; NHLBI, Epidemiology and Biometry Program; NIADDK; NIMH; and NIAAA.

LAB/BRANCH

Epidemiology Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.20	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to design and complete a followup of persons examined in the HANES I to study how factors previously measured relate to the health conditions that have developed since the survey. The three major areas for prediction of outcome are 1) nutrition 2) risk factors for chronic disease and 3) health care utilization. The survey will have a household interview including self-reporting of health conditions, utilization of health servics and behaviorial and social status plus some physical measurements as blood pressure, height, and weight. A feasibility study has been successfully completed to access cooperation. Tracing of individuals has begun and the pretest is to be completed in June 1981.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives/Issues Addressed: Purpose of this project is to design and complete a followup study of persons examined in the HANES I to study how factors previously measured relate to the health conditions that have developed since the survey. The major areas for prediction of outocme are nutrition, risk factors for chronic diseases, and health care utilization.

Approach/Methodology: Household interviews including self-reporting of health conditions, utilization of health services, and behavorial and social status plus some physical measurements as blood pressure, height, and weight will be completed on the original sample. A followup of all details and hospitalizations since the first survey will be included.

Significance to Biomedical Research/Justification: The HANES I sample is a representative sample of the noninstitutionalized U.S. population. Analysis of the results will be a screening of the physical, psychological and social areas as well as the utilization of health services to identify and define apropriate hypotheses for further investigation. Through the extensive information obtained by this followup study, indepth research studies can be designed to answer specific questions of interest to NIA.

Current Status/Major Findings: A feasibility study has been completed to assess the cooperation to be expected on the followup. Approximately 92 percent of the original population was located; 17 percent has died. Tracing of all 14,400 participants began January 1981; pretest to run June 1981; interviewing to begin October 1981 and continue through October 1983. Hospital data collection and death certificate collection will continue throughout the study period.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 02031 02 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Impact of Living Arrangement and Family Structure on the Health of the Elderly

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joan Cornoni-Huntley, Ph.D.	Epidemiologist	EDBP	NIA

COOPERATING UNITS (if any)

Division of Analysis, NCHS

LAB/BRANCH

Epidemiology Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study health, social, and economic problems of the elderly related to different aspects of residential living and family composition. In particular, families including elderly persons who are functionally disabled will be compared with other types of families with and without elderly persons.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: The purpose of this project is to study health, social, and economic problems of the elderly related to different aspects of residential living and family composition. In particular, families including elderly persons who are functionally disabled will be compared with other types of families with and without elderly persons.

Approach/Methodology: Information from the Health Interview Survey for multiple years will be combined to construct a computerized population. This population will be organized by households and families which will enable hypothesis to be tested for within and between family differences.

Significance to Biomedical Research/Justification: Elderly persons live in different residential arrangements either by choice or by necessity, resulting in either favorable or disadvantaged circumstances and outcomes. This study will attempt to predict the factors leading to beneficial outcomes of various living conditions.

Current Status/Major Findings:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 02050 01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Systolic Hypertension in the Elderly (SHEP)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joan Cornoni-Huntley, Ph.D.	Epidemiologist	EDBP	NIA
OTHERS:	William Friedwald, M.D.			NHLBI

COOPERATING UNITS (if any)
NHLBI

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Determine whether blood pressure lowering therapy reduces the rate of mortality from all causes of the occurrence of specified nonfatel morbid events in males and females 60 years of age and older with predominately systolic hypertension. The study is to evaluate whether treatment of isolated or predominately systolic hypertension in the elderly would decrease mortality.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives/Issues Addressed: Determine whether blood pressure lowering therapy reduces the rate of mortality from all causes of the occurence of specified nonfatel morbid events in males and females 60 years of age and older with predominately systolic hypertension. The study is to evaluate whether treatment isolated or predominately systolic hypertension in the elderly would decrease moratlity.

Approach/Methodology: NIA and NHLBI are equally cosponsoring a pilot study for a clinical trail of isolated systolic hypertension. The pilot study commenced in June 1980 and will run for 4 years. Participants in the study must be 60 years of age and over and have a diastolic blood pressure of 90 or below and a systolic blood pressure of 160 or higher. Each center will screen between 5,000 and 10,000 age-eligible individuals to obtain 100 study subjects. Thus, the entire pilot study will include 500 participants.

Significance to Biomedical Research/Justification: A large number of epidemiologic studies have shown that moderate and severe hypertension are related to increased mortality and cardiovascular renal disease morbidity. It is known that blood pressure continues to rise from 50 up to at least the age of 70 for both males and females. Several studies have shown that anti-hypertension therapy reduces that mortality in cardiovascular disease in persons less than 60 years of age who have moderate or severe hypertension. Information does not exist on the value of anti-hypertension therapy in the elderly. There is a need for additional evaluation in the elderly. The NHS estimated tha that 23 percent of males and 35 percent of females over age 60 have predominately systolic hypertension. It is not known whether anti-hypertension therapy significantly prolongs or improves the lines of the 8.6 million persons over 60 years of age. There is some uncertainty of the benefit of treatment in this group as well as some likelihood of treatment related harm. The proposed study will evaluate the use of anti-hypertension therapy in the age group 60.

Current Status/Major Findings: The actual screening will commence on April 1, 1981. Currently protocols are being developed for the various facets of the study and the staffs at the five institutions are being trained and procedures standardized. Screening will last for 15 months until June 1982 and then the 500 patients will be randomized and followed through June 1983.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 02062 01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Patterns of Use of Prescription Drugs: Sleeping Medications

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lon R. White, M.D., M.P.H.	Medical Epidemiologist	EDBP	NIA
OTHER:	Jacob A. Brody, M.D.	Associate Director for EDBP	EDBP	NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Examination of data collected by IMS, America, Inc., to describe patterns of concurrent prescription and related frequencies according to age and diagnosis. Work is in preliminary stages at present time.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: To describe patterns of hypnotic prescription by age, sex, major diagnosis, season, concurrent prescription, etc.

Approach/Methodology: Utilize data already collected by a commercial source (IMS America, an established NIH contractor)

Significance to Biomedical Research/Justification: In addition to providing important direct information concerning the consumption of these drugs, rates may also reflect rates of sleep disturbance morbidity. Such patterns may also be useful in the testing of hypothesis attempting to link disturbed sleep with the pathogenesis or early neurophysiologic changes of senile dementia.

Current Status/Major Findings: None as of April, 1981; initiating investigator has since left NIA; to be restarted in FY82 under another EDBP staff member.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 02090 03 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

General Analysis of National Center for Health Statistics Data Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joan Cornoni-Huntley, Ph.D.	Epidemiologist	EDBP	NIA
OTHERS:	Dennis Cosmatos, M.S.	Statistician	EDBP	NIA
	Jennifer Madans, Ph.D.			NCHS
	Joel Klienman, Ph.D.			NCHS

COOPERATING UNITS (if any)

Division of Analysis, NCHS

LAB/BRANCH

Epidemiology Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.10	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This interagency agreement allowed NIA to work cooperatively with NCHS in conducting analyses of appropriate data sets. The research topics included: (1) the relationship between water fluoride level and osteoporosis and (2) a description of the differentials in the geographical variation in stroke and heart disease mortality. These differentials are then related to geographic variations in selected risk factors.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: The purpose of this project was to provide analysis of NCHS data to answer specific hypotheses related to the elderly. The research investigation included: 1) the relationship between water fluoride level and osteoporosis, and 2) a description of the differentials in the geographical variation in stroke and heart disease mortality. These differentials are then related to geographic variations in selected risk factors.

Approach/Methodology: The analyses were to include a clear statement of the problem; identification of the appropriate data sets; determination of the best statistical methods for analysis; preparation of work tapes; programming and processing of data analysis, and the interpretation of the results and generation of further hypotheses.

Significance to Biomedical Research/Justification: Obtaining estimates on incidence, prevalence, change in these statistics and associations for the U.S. population for problems such as osteoporosis, and stroke are a contribution to biomedical research. Continually questions are generated and information is needed pertaining to these problems of the aged. This mechanism of obtaining answers from within the National Data System was extremely useful.

Current Status/Major Findings: We have a Preliminary Report on the Osteoporosis-Fluoride Project using HIS Data. This report is being reviewed internally at NCHS, will be submitted for NIA review, and possible publication in fiscal year 1982. NCHS will continue to analyze the stroke data.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 04001 01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Analysis of Functional Disability Data From Framingham Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lon R. White, M.D., M.P.H.	Medical Epidemiologist	EDBP	NIA
OTHERS:	Jacob A. Brody, M.D	Associate Director for EDBP	EDBP	NIA
	Joan Cornoni-Huntley, Ph.D.	Epidemiologist	EDBP	NIA
	Manning Feinleib, M.D. DrPh	Associate Director for E&B	EBP DHVD	NHLBI
	Robert J. Garrison	Chief, Biometrics Research Branch	EBP DHVD	NHLBI
	A. Jette, M.D.	Boston University, School of Medicine		
	L. Branch, M.D.	Boston University, School of Medicine		

COOPERATING UNITS (if any)
NHLBI

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Disability status of approximately 2800 surviving Framingham Heart Study participants was assertained. First description of the population is in press. Additional data analyses are currently in progress.

PROJECT DESCRIPTION:

Objectives Issues Addressed: The purpose of this study is to assess functional disability as it related to the problems of the aged among the Farmingham cohort. The questions to be answered within this analysis are 1. the prevalence of functional diability among the cohort, 2. the types of disability reported, 3. degrees of disability of the members of the cohort, and 4. determination of influence of previous physical conditions as predictors of prevalence.

Approach/Methodology: The analysis will be accomplished in three levels - 1) Dr. Lawrence Branch, who developed and administered the questionnaire to the Farmingham Cohort will clean the tapes, develop scores and produce descriptive statistics, 2) the disability data will be merged with the longitudinal data set 3) analysis of data by NIA in collaboration with NHLBI.

Significance to Biomedical Research/Justification: Provide information on the relationship between both previous and present health status of individuals and the presence, type, and degree of functional disabilities.

Current Status/Major Findings: Data collection has been completed and the first analysis/description (NHLBI authorship) is submitted for publication. NIA investigators are currently involved in additional analyses of these data.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 04003 01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Dementing Illnesses and Factors Associated With Nursing Home Admission in the Framingham Heart Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lon R. White, M.D., M.P.H.	Medical Epidemiologist	EDBP	NIA
OTHERS:	Robert Garrison, M.S.	Chief, Biometrics Research Branch	EBP DHVD	NHLBI
	M. Feinleib, M.D., DrPh	Associate Director for EBP	EBP DHVD	NHLBI
	Jacob A. Brody, M.D.	Associate Director for EDBP	EDBP	NIA

COOPERATING UNITS (if any)
NHLBI

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Framingham Study population is being utilized for studies on the prevalence, incidence, outcome, cause, and diagnostic categorization of dementia in the elderly.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: Carry out data collecting, processing, and analysis to accomplish: 1. Followup of approximately 160 patients previously identified as demented; to determine diagnosis and outcomes. 2. Case-control study of 50-80 patients with diagnosis of senile dementia; Alzheimer's type (SDAT); to search for risk factors associated with the disease (i.e. etiology). 3. Identification of "new" cases of SDAT which have become clinically apparent since tests conducted approximately 3 1/2 years ago to facilitate estimation of disease incidence (by age and sex), and to search for subtle changes in cognitive functioning which might have antedated the clinical onset of dementia. 4. A study of descriptive epidemiology of nursing home admission; with emphasis on dementia, incontinence, hip fracture, bedsores, and congestive heart failure.

Approach/Methodology: This will be a cooperative effort involving EDBP and NHLBI investigators. It will utilize data already collected and data which will be collected during the next 2 years as part of the ongoing study.

Significance to Biomedical Research/Justification: The nature of the study provides a unique opportunity to investigate both the cause and course of senile dementia.

Current Status/Major Findings:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 04010 03 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Catchment Area Study of Senile Dementia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joan Cornoni-Huntley, Ph.D.	Epidemiologist	EDBP	NIA
OTHERS:	William Eaton, Ph.D.			NIMH
	Ben Locke			NIMH

COOPERATING UNITS (if any)
National Institute of Mental Health
Center for Epidemiologic Studies

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .05	PROFESSIONAL: .05	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In conjunction with NIMH, NIA is completing a study of senile dementia in an adult community population., A contract has been awarded to Yale University to survey a sample of the New Haven, Connecticut adult population for this purpose. NIA is specifically interested in defining the occurrence of senile dementia in persons 65 years of age and older. The study will consist of a household interview and a followup of all subjects. A questionnaire has been developed by the investigators and the survey is currently in progress.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives/Issues Addressed: The Division of Biometry and Epidemiology, National Institute of Mental Health (NIMH) is in the process of investigating the prevalence of mental illness in an adult community population, Epidemiology Catchment Area Study (ECA). An award has been made to Yale University to survey a sample of the New Haven, Connecticut adult population for this purpose. The National Institute on Aging (NIA) is specifically interested in defining the prevalence of senile dementia in persons over 65 years of age.

Approach/Methodology: In addition to the household sample of 3000 in the original ECA Research design for the New Haven site, persons 65 years of age and older have been selected for interviews. Therefore, at least two household interviews will be completed on 3,000 persons representing the adult (over 18) population in the catchment area, and an additional 2,000 representing the aged (65 and over).

Significance to Biomedical Research/Justification: As more people age more people will develop senile dementia. Prevalence among those over 65 years of age with severe progressive disease had been found in different studies to range from 1 to 9 percent and up to 15 or 20 percent from milder forms. No epidemiologic patterns for senile dementia has been discerned by race, sex, geographic, occupation, environmental or infectious exposures. This project will attempt to provide epidemiologic patterns for etiologic clues.

Current Status/Major Findings: Household interviews have been completed on 2000 elderly persons. The second visits to some participants have begun. Data on a subsample of the population is being analyzed and six papers are to be presented at the 1981 American Psychiatric Assoc. Meetings. Staff Evaluation in July 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 04011 03 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Senile Dementia: Natural History in a Noninstitutionalized Population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joan Cornoni-Huntley, Ph.D.	Epidemiologist	EDBP	NIA
OTHERS:	Jacob A. Brody, M.D.	Associate Director for EDBP	EDBP	NIA
	Elizabeth Parker, Ph.D.			NIAAA

COOPERATING UNITS (if any)

National Institute on Alcohol Abuse and Alcoholism

LAB/BRANCH

Epidemiology Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .10	PROFESSIONAL: .10	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Epidemiologic studies of senile dementia are needed in order to determine if the disease occurs in specific patterns by age, sex, race, or other demographic and social characteristics and if such patterns would provide etiologic clues. This project will characterize persons within a community based, noninstitutionalized sample who appear to have senile dementia or to be at high risk of having this disorder. This sample will be compared with an unaffected group. Both samples will be followed over a three year period to discover risk factors and etiologic events.

PHS-6040
(Rev. 10-76)

PROJECT DESRIPTION:

Objectives/Issues Addressed: This study is to provide an indepth assessment of a community-based sample of persons who have been previously classified as either having senile dementia and/or depression or to be at high risk for having these disorders. The study includes a clinical examination plus a surveillance by contacting the subject and a close relative or friend every 9 months. Comparison will be made with ineffected persons to define risk factors and etiological events.

Approach/Methodology: This is a 5 year study of a free living population having been identified as positive for senile dementia and/or depression by a standard screening instrument. The cases and controls will have a clinical examination with valid and quantified measurment techniques and interviews every nine months to obtain information on demographic and social-psychological, cognitive functioning, drug usage, health service utilization, and other related factors. Similar information will be gathered from a relative or close friend.

Significance to Biomedical Research/Justification: Little is known about the occurrence of senile dementia in persons over 65 years of age. Etiologic mechanisms and risk factors are unknown; the natural history of this illness is obscure. Senile dementia and depression are apparently the most common disorders which primarily affect mental abilities in the elderly. Moreover, the two disorders may be indistinguishable. The project will provide a better definition of senile dementia and identify associated risk factors.

Current Status/Major Findings: Following the issuance and evaluation of a sources sought, an RFP was published producing a response of seven proposals. Four proposals were eliminated as not acceptable on first review and two after the second review.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 04014 02 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Epidemiologic Studies of Alzheimer's Senile Dementia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis Cosmatos, M.S.	Statistician	EDBP	NIA
OTHERS:	Peter Greenwald. M.D.	Health Research, Inc.		

COOPERATING UNITS (if any)
Health Research, Inc., New York State Department of Health
Division of Epidemioloy

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .05	PROFESSIONAL: .05	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aimed at the development of instruments, diagnostic criteria, and procedures necessary to conduct a planned, geographically based epidemiologic study of Alzheimer's Senile Dementia. With the help of staff from Health Research, Inc., methods to identify patients with senile dementia of the Alzheimer type (SDAT) were developed. Examination of the criteria most critical in the diagnosis of SDAT patients was made. The reliability and validity of the procedures developed was determined.

PROJECT DESCRIPTION:

Objectives/Issues Addressed: NIA, EDBP, worked with members of Health Research, Inc., the New York State Department of Health in the development of methods to establish a diagnosis of Senile Dementia of the Alzheimer's type (SDAT) in a general research setting. These methods attempted to resolve the differences between the "ideal" approach that is feasible only in a university hospital and realities of a community-wide study. There was an examination to determine which criteria should be assessed in making the diagnosis of Alzheimer's Senile Dementia (e.g., memory abstract thinking, personality) and developing a procedure to translate diagnostic criteria into a final statement of diagnosis. Methods for checking the reliability of the diagnosis were developed. The methods included a plan for efficient use of clinicians in making and validating the diagnosis in a large group of patients.

Approach/Methodology: An expanded and current literature review of Alzheimer's Senile Dementia; mental status tests and related topics were reviewed. Methods being used in current epidemiologic research studies of Alzheimer's Senile Dementia were reviewed and a set of diagnostic criteria and a protocol was developed for diagnosis of Alzheimer's Senile Dementia in community-based epidemiologic study. The protocol was a pilot test for feasibility and reliability.

Significance to Biomedical Research/Justification: In any epidemiologic study, it is understood that the identification of the cases are of paramount importance. Any associations detected between the condition of interest and secondary factor, is contingent on the accurate identification of the subjects with the test condition. This project was reviewed and all procedures presently employed for the identification of SDAT cases and compiled a single comprehensive diagnostic procedure high in reliability and validity, to be standardly applied by researchers in this area.

Current Status/Major Findings: A final report has been received. Staff evaluation of this report has been unanimously laudatory; we expect this document to serve as the foundation of our prospective studies on dementia for the next several years.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 040501 01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
A Study of the Last Days of Life

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas A. Parker, Ph.D.	Sociologist	EDBP	NIA
OTHERS:	Dean Echenberg, M.D.	University of California, Berkeley		

COOPERATING UNITS (if any)
University of California, Berkeley

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This professional services contract is in support of the first in a series of projected epidemiologic studies of the last days of life. Because the great majority of deaths occur among the aged and the events and circumstances immediately preceeding death are poorly understood, NIA is concerned with the development of systematic information on the last days of life from spouses or relatives who had personal knowledge of a representative sample of deceased persons and from attending physicians who had medical knowledge of the deceased.

PROJECT DESCRTIPTION:

Objectives/Issues Addressed: This professional services contract is in support of the first in a series of projected epidemiologic studies of the last days of life. Because the great majority of deaths occur among the aged and the events and circumstances immediately preceeding death are poorly understood, NIA is concerned with the development of systematic information on the last days of life from spouses or relatives who had personal knowledge of a representative sample of deceased persons and from attending physicians who had medical knowledge of the deceased.

Approach/Methodology: A professional services contract with a medical epidemiologist at the University of California, Berkeley, was initiated during the fall of 1980 in order to produce a questionnaire for pilot testing in studies of the last days of life.

Significance to Biomedical Research/Justification:

Current Status/Major Findings: It is expected that Dr. Echenberg's final report will be submitted by summer of 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 05001 01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Congestive Heart Failure and Digitalis Use

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lon R. White, M.D., M.P.H.	Medical Epidemiologist	EDBP	NIA
OTHERS:	Jacob A. Brody, M.D.	Associate Director for EDBP	EDBP	NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is in a preliminary stage. Involves analysis of data available from IMS America, Inc., for demographic and concurrent prescription patterns for digitalis.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives/Issues Addressed: To describe patterns of digitalis use among the elderly.

Approach/Methodology: Information is obtained from a commercial source (IMS America, an eatablished NIH contractor) and is analyzed with special attention to age, sex geographic area, diagnosis, concurrent drug use, and certain other variables.

Significance to Biomedical Research/Justification: Preliminary data suggest that the incidence and prevalence of digitalis use far exceed the incidence and prevalence of congestive heart failure, the principal indication for its use. Survival prognosis appears likewise to be very different for initiation of digitalis treatment as compared with the prognosis for heart failure. Possible explanations for these discrepancies include: 1. the statements are untrue; 2. digitalis is overprescribed; 3. congestive heart failure is underdiagnosed. A resolution of the question is of great importance because of the magnitude of the problem and because it might well modify current prescribing practices.

Current Status/Major Findings: Preliminary analysis of the age distribution of digitalis recipients substantiates the impression that most of the prescriptions are written for older people. Although precise age-specific incidence and prevalence figures are not yet available, those data which are available seem to suggest that these rates double about every 7-9 years of age, thus resembling other phenotypic manifestations of aging.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 07010 01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Relationship Between Childhood Growth and Adult Habits, Morbidity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lon R. White, M.D., M.P.H.	Medical Epidemiologist	EDBP	NIA
OTHERS:	Mary E. Farmer, M.D.	Epidemiologist Trainee	EDBP	NIA
	George E. Comstock, M.D.	Johns Hopkins University, School of Hygiene and Public Health		

COOPERATING UNITS (if any)
The Johns Hopkins University, School of Hygiene and Public Health

LAB/BRANCH
Epidemiology Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Existing data from Washington County Health Study reviewed to determine feasibility of a larger study. In this preliminary study, no clear relationship observed between childhood habitus and adult blood pressure.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: The questions addressed are: 1. does childhood habitus predict adult habitus, 2. are certain childhood habitus or growth characteristics associated with cardiovascular disease patterns, 3. are childhood growth patterns associated with longevity or with physiologic aging rate?

Approach/Methodology: The principal source of data will be the Hagerstown/Comstock/Hopkins study. A feasibility study of approximately 600 individuals originally examined between 1925-1940 (at about age 10 years) will indicate to what extent these data can be applied to the questions above. Subsequent course will be determined by this preliminary analysis.

Significance to Biomedical Research/Justification: The questions listed are of fundamental importance to our understanding of the role of health and nutrition in childhood in determining health in adult life.

Current Status/Major Findings: A feasability study was conducted as planned. No clear relationship was observed between childhood relative weight and adult blood pressure; however, the numbers were too small to draw any definite conclusions. The study demonstrated that data on health resulting from a blood pressure survey done 6 years ago is available for approximately 20 percent of the individuals for whom childhood growth data are available. Based on the known 33 percent community participation in the recent survey, it is estimated that approximately 50 - 60 percent of the original study participants are still alive and living in Washington county. This study was carried out under the supervision of Dr. George Comstock and with the assistence of Dr. Mary Farmer.

Contract Number: N01-AG-0-2105

Contract Title: Establishment of Populations for Epidemiologic Studies of the Aged

Contractor: Yale University

FY 81 Funding: $558,830.

Objectives/Issues Addressed: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged. Studies are to be completed on problems of pain, vision, hearing, sleep, drug use, constipation, social support and other pertinent areas.

Approach/Methodology: The project shall include cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population will be included.

Significance to Biomedical Research/Justification: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. It is important to have studies representing existing conditions in a community population. Within obvious logistical contraints populations will be available to the NIA scientific community for specific studies. High priority short term studies will be encouraged.

Current Status/Major Findings: During the first year, the demographic and social characteristics of each community have been reported. Also, a description of available health care facilities has been prepared. Extensive work is being done on development of the baseline survey. A section of this survey which includes information on chronic conditions, and social, demographic and psychological characteristics will be common to all centers. Pilot testing of this instrument is expected in June and July 1981 and will be followed by completion of the baseline survey.

Publications: None to date.

Contract Number: N01-AG-0-2106

Contract Title: Establishment of Populations for Epidemiologic Studies of the Aged

Contractor: University of Iowa

FY 81 Funding: $150,000.

Objectives/Issues Addressed: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged. Studies are to be completed on problems of pain, vision, hearing, sleep, drug use, constipation, social support and other pertinent areas.

Approach/Methodology: The project shall include cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population will be included.

Significance to Biomedical Research/Justification: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. It is important to have studies representing existing conditions in a community population. Within obvious logistical contraints populations will be available to the NIA scientific community for specific studies. High priority short term studies will be encouraged.

Current Status/Major Findings: During the first year, the demographic and social characteristics of each community have been reported. Also, a description of available health care facilities has been prepared. Extensive work is being done on development of the baseline survey. A section of this survey which includes information on chronic conditions, and social, demographic and psychological characteristics will be common to all centers. Pilot testing of this instrument is expected in June and July 1981 and will be followed by completion of the baseline survey.

Publications: None to date.

Contract Number: N01-AG-0-2107

Contract Title: Establishment of Populations for Epidemiologic Studies of the Aged

Contractor: Peter Bent Brigham Hospital

FY 81 Funding: $433,343.

Objectives/Issues Addressed: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged. Studies are to be completed on problems of pain, vision, hearing, sleep, drug use, constipation, social support and other pertinent areas.

Approach/Methodology: The project shall include cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population will be included.

Significance to Biomedical Research/Justification: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. It is important to have studies representing existing conditions in a community population. Within obvious logistical contraints populations will be available to the NIA scientific community for specific studies. High priority short term studies will be encouraged.

Current Status/Major Findings: During the first year, the demographic and social characteristics of each community have been reported. Also, a description of available health care facilities has been prepared. Extensive work is being done on development of the baseline survey. A section of this survey which includes information on chronic conditions, and social, demographic and psychological characteristics will be common to all centers. Pilot testing of this instrument is expected in June and July 1981 and will be followed by completion of the baseline survey.

Publications: None to date.

Overview of Demographic and Economics Branch

The Demographic and Economics Branch studies the social, economic, and biomedical phenomena involved in population and individual aging. One concern is how the economic behavior and productivity of the U.S. economy responds to population aging. Both the mechanisms of aging at the individual level and the change in the numbers of elderly who compose the population influence the response of the economy to population aging. Created by population aging, this concern is sometimes referred to as the problem of increasing "economic dependency." A second concern is with the well being of the elderly, both in the individual and aggregate sense. The complicated interactions of per capita national income, private and public pensions, government transfers, the health and functional ability of the elderly, the ability and propensity to work, and the provision of general and long-term health care all contribute to the elderly's standard of living and sense of well being.

The Demographic and Economics Branch has, through the mechanism of an Interagency Agreement with the President's Commission on Pension Policy, brought into existence the nation's first demographic-macroeconomic growth model of the U.S. retirement income system. It has already been used by the President's Commission in its analytical policy research concerning a new restructuring of the retirement income system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01050 02 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Age Structure in a Macroeconomic Model of the U.S. Economy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William S. Cartwright, Ph.D.	Economist	EDBP	NIA

COOPERATING UNITS (if any)

President's Commission on Pension Policy

LAB/BRANCH

Demography and Economics Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A Demographic-Macroeconomic Model of the U.S is being developed. The impact of population aging will be examined on the nation's economy, retirement income system, labor force, and the welfare of the aged. The Demographic Macroeconomic Model represents the integration of eight models - Hudson, Jorgenson Growth Model, Anderson Labor Model, ICF Inc. Population Model, Social Security Model, Private and Public Employees Pension Model, Supplemental Security Income Model, and Medicare Model.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives/Issues Addressed: To determine the impact of aging on the U.S. economy and the impact of economic changes on the elderly segment of the population.

Approach/Methodology: The Hudson-Jorgenson macroeconomic growth model of the U.S. is being combined with a demographic projection model as well as others to examine age and sex population and labor force participation, income, and total output. This model is a computerized system of mathematical equations set up on the NIH computer. The mathematical equations are based on statistical estimates and specific assumptions about legal and institutional relations in the economy. the computer simulation program permits projections of demographic and economic variables.

Significance to Biomedical Research/Justification: The model will aid all NIA programs to assess the size and economic conditions of the elderly population. This specifically relates to the problems of population aging, and social behavioral responses, and biomedical induced changes in the length and quality of life.

Current Status/Major Findings: A model has been statistically estimated and an operating simulation program developed. The President's Commission on Pension Policy is using the current model for analytical purposes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01230 02 EDBP

PERIOD COVERED
October 1, 1980 to December 30, 1980

TITLE OF PROJECT (80 characters or less)
Demographic Patterns of Mortality

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Clifford H. Patrick, Ph.D.	Research Demographer	EDBP	NIA
OTHERS:	Yuko Y. Palesch, M.S. , NIA	Research Statistician	EDBP	NIA

COOPERATING UNITS (if any)
College of Urban Affairs and Public Health
University of Delaware

LAB/BRANCH
Demography and Economics Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
National Institute on Aging, NIH

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Selected patterns of interstate migration for the elderly population of the U.S. are analyzed using demographic methodology. First, a descriptive analysis is conducted and the exploratory behavioral analysis using preference indexes and gravity models.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues addressed: An analysis of migration patterns of demographic segments of the elderly population of the U.S.

Approach/Methodology: Professional Services Contract

Significance to Biomedical Research/Justification: Unique data set available only at the University of Delaware School of Public Policy

Current Status/Major Findings: Closed.

Overview of Biometry Branch

The Biometry Branch in concerned with the mathematical, statistical, and numerical aspects of the various activities of the EDBP and exercises responsibility in devising, developing, and modifying methodologies in these areas for use in medical research programs. In addition, a biometry research program is being developed to complement and interact with the the research programs being carried out in the epidemiology and demography and economics branches. Fiscal year 1981 has been a year of change and redirection in our program with two new staff members in biometry having joined the group within the last 3 months. While much of our time has been spent becoming better acquainted with the Institute and the Program, and in planning our program, we have been involved in our own ongoing research as well.

Our study of the relationship between climatic extremes and mortality rates among the elderly continues. We have received data tapes from the National Climatic Center covering 30 years, and preliminary processing of the data on those tapes had been successfully completed. Similiarly, mortality data tapes from NCHS have undergone successful preliminary processing. We are now seeking means by which both sets of data can be put on the same geographic basis. It is likely that this geocoding problem can be at least partially solved by the use of computer files which have already been developed. Then we can proceed with our investigation which, in addition to climatic data, may also include air and water quality and other environmental data available from the Council on Environmental Quality (CEQ), the latter being obtained through the User Prompted Graphic Data Evaluation (UPGRADE) System. Other aspects of this project include the possible removal of secular trends in mortality rates through the use of time-series methods before analysis and an investigation of the lag time between occurrence of climatic extremes and mortality.

We are consolidating and expanding studies of trends and differentials in mortality by cause, age, sex, and race. Previous work considered sex differentials in cohort mortality from cardiovascular disease. We are examining mortality from the five leading causes of death for various age, sex, and race groups, and over time. To date we have seen that, among the elderly population, deaths from cardiovascular disease tend to parallel the total death rate by age and constitute the major proportion of the total death rate. This pattern has continued over several recent years for which we have data. Our efforts to study mortality among the 85 years and older population have been enhanced by our recent use of more detailed Census population estimates accessible now through the Michigan Data Archive which is supported by an NIA interagency agreement through AoA. The use of such population contracts will become more and more important as larger numbers of persons survive into the older ages in the future.

Drs. Brody and Brock have been asked to collaborate in the writing of a chapter on the Epidemiology of Aging to be a part of the second edition of the Handbook of the Biology of Aging to be published in 1982 by Van Nostrand Reinhold. Some of the results of the studies on demographic and mortality trends will be included as part of this effort.

Another area of interest among our group is length of stay in nursing homes and related issues. A study which led to a method of determination of length of stay in nursing homes was completed collaboratively with the Health Care Financing Administration. We are following this with an analysis of the newly- determined length of stay among residents of nursing homes with chronic conditions. The data source for this investigation will be the 1977 National Nursing Home Survey of NCHS. A variety of characteristics of nursing homes will be examined to study possible relationships with length of stay of those persons requiring care for the leading chronic conditions. The study could be expanded in the future to include other subpopulations of interest within nursing homes, for example, persons with mental impairments.

We have renewed our interagency agreement with Jacob Siegel at the Bureau of the Census to continue special analysis of the latest available data from the Census on the elderly population. We are expecting an update to the report "Social and Economic Characteristics of the Older Population: 1978." In addition the agreement is supporting additional research on mortality projections, a special report on the characteristics of the mid-life population (ages 40 to 64), and initiation of work on an annotated bibliography of aging and the design of special tabulations based on the 1980 Census. In other Census related activities, we have proposed an interagency agreement to support the early production of a public use sample for aging research based on the 1980 Census. This follows the recommendations received in the report from the professional services contract with the University of Miami in FY80.

A professional services contract with Dr. Benjamin Bradshaw of the University of Texas School of Public Health has been completed. This project was to provide life table analysis of the Hispanic population at three levels of geographic detail within the state of Texas. Dr. Bradshaw's report is being forwarded to EDBP.

A report has been received from Texas A&M University on a professional services contract which provided an evaluation of the Aging Research Information System (ARIS) run by that university. The system appears to be somewhat out-of-date and would require considerable additional funding to maintain. Staff evaluation recommended no further investment in this system at the present time.

A study of interstate migration of the elderly based on 1970 Census data has been completed. This project was funded by a professional services contract with Dr. Norfleet W. Rives at the University of Delaware. Findings from the study indicated that interstate migration tends to increase with age up to the 65 to 69 year age groups and then decreases. As expected, the migration streams are dominated by females and the direction of movement is toward the south and southwest and away from the northeast and midwest. No further followup on this study is planned at this time.

A project which had been proposed on a comparative study of aging of the Japanese-American population in Hawaii and the U.S. population has been discontinued. The staff member who proposed the project has left the Program and nothing further is planned at this time.

A mortality followback survey had been planned as an interagency agreement with NCHS. The purpose was to determine the status of the deceased in their last days of life and to study differences between survivors and nonsurvivors with respect to their socioeconomic and demographic characteristics. However, because of severe personnel limitations at the NCHS, we have not been able to execute this interagency agreement.

Two other areas in which we plan to devote some attention involve analysis of currently available data in preparation for the subsequent arrival of data from our population studies and from the HANES Followback. First, information from HANES I on nutrition (including dietary intake, food composition and biochemistry) will be analyzed to describe the status of the population at the time of the first examination. The second area involves the study and evaluation of a process model of morbidity and mortality developed by Professor Max Woodbury of Duke University for assessing chronic disease among the elderly when risk is known to change over time. This may have possible application to our population studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01070-01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Public Use Sample (1980 Census) for Aging Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles F. Longino, Jr., Ph.D. Department of Sociology
University of Miami, Coral Gables, Florida 33124
OTHERS: Norfleet W. Rives, Jr., Ph.D. University of Delaware
Jeanne C. Biggar, Ph.D. University of Virginia

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
This project provided a proposal for the design and development of a research oriented public use sample data file from the 1980 U.S. Census of the elderly population. Technical specifications, including sample sizes, assignment of sampling weights, universe description, geographic coverage, and statistical reliability were given.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: Design and develop research oriented public use sample data file from the 1980 U.S. Census of the elderly population.

Approach/Methodology: Feasibility for the design was established in report received from Professional services contract with University of Miami. Sample will be designed and selected by the U.S. Bureau of the Census, Data Users Services Division.

Significance to Biomedical Research/Justification: Data from the 1980 Census are available only from the U.S. Bureau of the Census.

Current Status/Major Findings: The contractor's report on the feasibility study has been received. The interagency agreement is under negotiation, subject to the availability of funds.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01090 02 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Demographic Aspects of Aging in the Older Population in the U.S.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dwight B. Brock, Ph.D.	Statistician	EDBP	NIA
OTHERS:	J. Seigel	U.S. Bureau of the Census		

COOPERATING UNITS (if any)

U.S. Bureau of the Census

LAB/BRANCH

Biometry Program

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The data will allow the impact of selective biomedical research which is age specific to be calculated for the elderly. The census analysis will indicate the magnitude of the elderly population and the growth in the size of the elderly population in all areas of the population in all areas of the nation by demographic and socioeconomic groups.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

Objectives/Issues Addressed: 1) To determine the nature of demographic aging of the U.S. population. 2) To obtain the latest available census information on the number and proportions of older people, their distribution and socio-economic characteristics. 3) To provide information on the methodologies used in analyzing the demographic data in the Census source to draw valid conclusions.

Approach/Methodology: 1) To update special studies series P-23 No. 59, entitled "Demographic Aspects of Aging in the Older Population of the U.S." 2) To examine in depth the various socio-economic categories of the elderly population and the minority population among the elderly. 3) To examine in detail the family structure and the demographics of minorities among the elderly.

Significance to Biomedical Research/Justification: The data will allow the impact of selective biomedical research which is age specific to be calculated for the elderly. The census analysis will indicate the magnitude of the elderly population and the growth in the size of the elderly population in all areas of the nation by demographic and socioeconomic groups.

Current Status/Major Findings: Work in progress. Draft report on social and economic characteristics of the mid-life population received.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01120 01 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

SSA Study of Awards to Retirees, Dependents and Survivors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Clifford H. Patrick, Ph.D.	Research Demographer	EDBP	NIA

COOPERATING UNITS (if any)

Social Security Administration

LAB/BRANCH

Demography and Economics Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .01	PROFESSIONAL: .01	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Social Security Administration will contract with outside firm(s) to conduct a field test with respect to planning the 1981 survey of 11,000 people concerning economic factors, human factors, and attitudes and behavioral aspects of work and retirement

PROJECT DESCRIPTION:

Objectives Issues Addressed: To engage in cooperative research with the Social Security Administration on the issues of work and retirement.

Approach/Methodology: Survey techniques related to field testing for planning a 1981 survey of 11,000 individuals.

Significance to Biomedical Research/Justification: Would have permitted an investigation into retirement and health factors related to the older population.

Current Status/Major Findings: Social Security Administration withdrew its support of this work until further development of design, content, and scheduling are completed. They will review their interest to collaborate at an unspecified later time.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01161 01 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Length of Stay in Determining Rehabilitative Care or Maintenance Care for Residents in Nursing Homes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dan Foley	Statistician	EDBP	NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To determine the relationship between LOS and the level of care, discharge, status , and costs for discharges from nursing homes. Also to determine what type of nursing facility has the highest proportion of long staying residents.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives/Issues Addressed: 1. To determine the relationship between LOS and the level of care, discharge status, and costs for discharges from nursing homes, 2. To determine what type of nursing facility has the highest proportions of long staying residents.

Approach/Methodology: Regression analysis on the discharge sample of the National Nursing Home Survey to determine what variables affect LOS. Regression analysis on the type of facility with long-staying residents.

Significance to Biomedical Research/Justification: Specific health conditions affect the LOS in nursing homes; however other characteristics of the resident as well as the facility type may futher influence the LOS. The information obtained from this analysis will increase our knowledge in this area.

Current Status/Major Findings: Ongoing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01250 02 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Michigan Data Archive - National Archive of Computerized Data on Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis Cosmatos, M.S.	Statistician	EDBP	NIA
OTHERS:	Ronald Ables, Ph.D.		SBR	NIA
	Michael Traugott, Ph.D.	ICPSR/University of Michigan		
	Frances Jacobs, Ph.D.	Administration on Aging		

COOPERATING UNITS (if any)
Inter-University Consortium for Political and Social Research, University of Michigan

LAB/BRANCH
Biometry Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .05	PROFESSIONAL: .05	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The National Archive of Computerized Data on Aging (NACDA) is being developed within the framework of the Michigan Data Archives as a joint funding effort between the Administration on Aging and National Institute on Aging. Data contained in NACDA can be accessed by intramural research staff at NIA and analyses performed using the University of Michigan computer facilities. By using the Telenet communication lines and remote terminals to link with the Michigan Terminal System, analyses involving data on the health, social, economic, and demographic characteristics of the elderly will be interactively performed.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives/Issues Addressed: To develop an archive of data relevent to the study of health and social problems encountered by the elderly.

Approach/Methodology: The National Archive of Computerized Data on Aging (NACDA) is being developed within the framework of the Michigan Data Archives as a joint funding effort between the Administration on Aging and National Institute on Aging. Data contained in NACDA can be accessed by intramural research staff at NIA and analyses performed using the University of Michigan computer facilities. By using the Telenet communication lines and remote terminals to link with the Michigan Terminal System, analyses involving data on the health, social, economic, and demographic characteristics of the elderly will be interactively performed.

Significance to Biomedical Research/Justification: The Michigan Data Archives is a system in use by a majority of the research and educational institutions in the United States. By developing an archive of data relevent to aging research more investigators will be able to access data which is necessary in addressing health and social problems which confront the elderly.

Current Status/Major Findings: Continuing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 01270 01 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Health Research Potential of the Aging Research Information System (ARIS)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yuko Palesch, M.D.	Statistician	EDBP	NIA
OTHERS:	Arthur Cosby, Ph.D.,	Office of University Research Office of Human Resources Research and Development, Texas A&M University College Station, TX 77843		
	Steven F. Phillip	Texas A&M University		
	Kenneth L. Nyberg	Texas A&M University		

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project provided an evaluation of the health research potential of ARIS, a 20,000 item bibliography on aging research collected under sponsorship of the Texas Department of Human Resources. The consistency and quality of data sources were evaluated and analyses of the uses of ARIS were included. Recommendations to EDBP resulting from evaluation of ARIS were provided.

PROJECT DESCRIPTION:

Objectives Issues Addressed: Description and assessment of research potential of the ARIS data.

Approach/Methodology: Professional Services Contract

Significance to Biomedical Research/Justification: This unique data set is available only at Texas A & M University

Current Status/Major Findings: The information in this report, while fulfilling the objectives of the scope of work of the contract, does not appear to offer a particularly valuable resource to EDBP. The most recent information in the bibliography is from 1977, with most of the entries being considably older. It is likely that similar information could be obtained from searches conducted by the National Library of Medicine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 02064 02 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Trend in Drug Use Among the Elderly Population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis Cosmatos, M.S.	Statistician	EDBP	NIA
OTHER:	Fred Eckel, M.S.	North Carolina Pharmaceutical Research Foundation		

COOPERATING UNITS (if any)

North Carolina Pharmaceutical Research Foundation (NCPRF)
Unversity of North Carolina at Chapel Hill

LAB/BRANCH

Biometry Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .05	PROFESSIONAL: .05	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In conjunction with the North Carolina Pharmaceutical Research Foundation, NIA is conducting a study of drug usage by the elderly.

The Medicaid records for years 1973 thru 1976 will be the primary data source for this study. Questions on self medication, medical care factors, drug cost, and drug problems related to the elderly will be addressed.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives/Issues Addressed: This study aims at identifying several aspects of drug usage in the elderly. These will include quantity and quality of drugs used, self medication, relationships of demographic factors and medical factors to drug usage, identification of the prescribers and dispensers of the drugs, relationships between drug cost and drug usage, drug related problems seen in the the elderly, and problems related to the use of specific drugs in the treatment of several chronic conditions.

Approach/Methodology: The Medicaid Data Records for years 1973 through 1976 will be the primary data source which will be used in addressing the questions presented above. Staff from the NCPRF have developed the data set so it is in a form which allows immediate application to pharmaceutical research

Significance to Biomedical Research/Justification: As a group which is more prone to illness, the elderly are therefore more subject to drug treatments. A detailed profile of drug usage may suggest cases of drug interaction, overusage, and underusage. Estimates of drug efficacy for specific conditions may also be feasible if the usage patterns identified by this study are compared to morbidity and mortality data. This will be the first study of this type to focus on the health aspects of drug usage in this group as a primary concern with marketing and cost aspects secondary.

Current Status/Major Findings: Extension of the contract to July 1, 1981, will be required.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 02080 01 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Secondary Analysis of HANES Predictive Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis Cosmatos	Statistician	EDBP	NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .05	PROFESSIONAL: .05	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
In preparation for the extensive follow-up survey for HANES I we anticipate completing preliminary analyses of the current baseline data. In particular the information on nutrition including dietary intake, food composition, and the biochemistry will be analyzed to describe the status of the population at the time of the first examination. Through these preliminary analysis subjects may be grouped into levels of nutritional intake or dietary behavior.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: In preparation for the extensive follow-up survey for HANES I we anticipate completing preliminary analyses of the current baseline data. In particular the information on nutrition, including dietary intake, food composition, and the biochemistry will be analyzed to describe the status of the population at the time of the first examination. Through these preliminary analyses subjects may be grouped into levels of nutritional intake or dietary behavior.

Approach/Methodology: The methods for analysis will be defined when the data is received and the hypotheses are clearly defined.

Significance to Biomedical Research/Justification:

Current Status/Major Findings:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 04013-02 EDBP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Relationship Between Down's Syndrome and Alzheimer's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis Cosmatos, M.S.	Statistician	EDBP	NIA
OTHERS:	Donna Cohen			

COOPERATING UNITS (if any)
University of Washington, Seattle, School of Public Health and Community Welfare, Department of Epidemiology

LAB/BRANCH
Biometry Branch

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	.05

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
This study is to investigate the prevalence of Alzheimer's Disease in relatives of individuals with Downs's Syndrome. Patterns of familial aggregation of these diseases will be compared to their genetic models. The study will focus on 200 probands.

PROJECT DESCRIPTION:

Objectives/Issues Addressed: There has been extensive speculation about the possible relationship between Alzheimer's Disease and Down's Syndrome. Several previous studies have demonstrated an excessive prevalence of Alzheimer's Disease, Down's Syndrome, leukemia and related disorders in the families of probands diagnosed to have Alzheimer's Disease. It is speculated that a common genetic diathesis underlay both disorders. Among the many important questions raised by these studies are the following: Is there an increased prevalence of Alzheimer's Disease in relatives of individuals with Down's Syndrome; and, is the pattern of familial aggregation of these diseases consistent with a genetic model? An affirmative answer could be required to provide positive evidence for the hypothesis of a common genetic diathesis.

Approach/Methodology: In order to answer these questions in an epidemiologically sound manner, a study has been designed which has probands cross matched for Alzheimer's Disease and Down's Syndrome as well as having an adequate group of control probands. This required study of the family members of the following four groups of probands, using a standardized procedure:

1. probands who have a child with Down's Syndrome
2. probands with Alzheimer's Disease
3. probands with a nondementing neurological disorder (e.g. Parkinson's)
4. probands representing a random sample of the population.

All probands being age and sex matched and information gathered about the prevalence of possible Alzheimer's Disease, and Down's Syndrome and related disorders in first degree relatives. This study will use data collected on 200 probands all of whom have at least one child with Down's Syndrome. Data on the increased prevalence of Alzheimer's Disease is suggested.

Significance to Biomedical Research/Justification: The relationship between Alzheimer's Disease and Down's Syndrome has been examined by numerous investigators. This, however, is the first study to undertake an active genetic-epidemiological approach to the relationship of these diseases. The identification of both diseases as being governed by the same genetic mechanism will lead the way to a better understanding of the conditions and eventually a better understanding of the underlying etiology of the diseases.

Current Status/Major Findings: A report is due September 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 06000 02 EDBP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Climatic Effects on Morbidity/Mortality in the Elderly

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis Cosmatos, M.S.	Statistician	EDBP	NIA

COOPERATING UNITS (if any)

National Climatic Center, NOAA

LAB/BRANCH

Biometry Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.40	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data on daily climatic conditions as reported from approximately 300 weather stations will be obtained from the National Climatic Center, and linked to daily mortality data for the period 1972 through 1977. An age-specific analysis of average daily mortality at various levels of climatic variables will be performed in order to ascertain relationships between age and climatic conditions. Climatic variables will include temperature, relative humidity, rapid changes in temperature, departures from normal temperatures, and sustained heat waves and cold fronts. Levels at which hypothermia and hyperthermia are more likely will be investigated in greater detail. Concomitant information on socioeconomic status and geographic location of the cases will also be considered.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: The basic aim of this project is to investigate responses to temperature extremes by people in different age groups, incorporating concomitant information on socio-economicc status, and geographic location. A basis hypothesis of older people living in relatively warmer climates with lower socioeconomic status being more susceptible to climatic extremes, will be addressed. Identification of the climatic conditions which effect the various age groups most will also be attempted. A variety of climatic variables and their interactions will be used.

Approach/Methodology: Daily climatic data will be purchased from the National Climatic Center and linked with detail mortality data collected by the National Center for Health Statistics. Since day of death has only been coded on the mortaltiy tapes for years 1962 through 1966 and 1972 through the present, the study of daily mortality is limited to these dates. Initially, only the most recent group will be examined. As the study proceeds, both groups will be examined and monthly analyses on the intermediate years will be performed.

Current Status/Major Findings: Research in progress. NCC data obtained by interagency agreement. IAA completed in FY 81.

Significance to Biomedical Research/Justification: Most studies of this type fail to examine responses to climatic variables across separate age groups. This study will investigate differences in age-specific mortality/morbidity at various climatic extremes. Factors such as financial conditions and geographic location will also be considered. It is expected that several new areas of interest will develop as the project progresses.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 06020 01 EDBP

PERIOD COVERED

March 31, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Health Effects of Environmental Pollutants on the Elderly (UPGRADE Project)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. Dennis Cosmatos, M.S.	Statistician (Health)	EDBP	NIA

COOPERATING UNITS (if any)

Presidents Council on Environmental Quality, Executive Office of the President

LAB/BRANCH

Biometry Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .10 (NIA)	PROFESSIONAL: .10 (NIA)	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations will be made into the effects of environment on the health of the elderly. Interactions of environmental pollutants with climate variables will be investigated with respect to their effects on a variety of specific chronic conditions and mortality.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives Issues Addressed: The effects of a variety of environmental pollutants on the health status of a group which is particularly susceptible to illness, namely the elderly, will be investigated. Effects on morbidity and mortality of the group will be examined.

Approach/Methodology: EPA data bases containing detailed exposure data on a variety of water and air pollutants will be linked with morbidity and mortality data on the elderly in order to identify associations between exposures to ambient environmental pollutants and health status. The UPGRADE software system will be used to perform analyses and display results of analyses in graphical or tabular form. The system will also be modified to accomodate climatic data purchased from the National Climatic Center.

Significance to Biomedical Research/Justification: Classic studies of health effects of pollutants focus on the general population and fail to address a possible increased sensitivity by the elderly to these pollutants. This study will specifically focus on the effects of these pollutants on the elderly. A variety of specific software capabilities not presently avialable on current computer systems will be devloped in order to perform the climate/environmental pollutant analyses.

Current Status/Major Findings: Research in progress.

GERONTOLOGY RESEARCH CENTER

Report of the Intramural Research Program

Office of the Scientific Director	GRC/OSD-1
Laboratory of Behavioral Sciences	GRC/LBS-44
Laboratory of Cellular and Molecular Biology	GRC/LCMB-133
Laboratory of Molecular Aging	GRC/LMA-160
Laboratory of Neurosciences	GRC/LNS-201
Clinical Physiology Branch	GRC/CPB-256

Gerontology Research Center

The Center's achievements this year are exemplified by the output of its scientific staff, which includes 70 research papers or chapters published during the first nine months of FY 1981. The results reported range from basic findings contributing to a fuller understanding of aging processes to those having direct or potential clinical application for the treatment of older patients. Sample highlights of this research follow.

The Laboratory of Behavioral Sciences's Geriatric Continence Clinic completed its first year of operation. Under the direction of Dr. Bernard T. Engel and Guest Worker, Dr. William E. Whitehead, the clinic provides free evaluation and treatment to outpatients experiencing either fecal of urinary incontinence. Patients are all 65 years of age or older and most of those seen in this clinic to date have successfully lowered their incidence of incontinence or have become totally continent. Clinic staff investigate why some older individuals become incontinent while, at the same time, evaluating behavioral training techniques with potential for treatment. Techniques currently in use include both operant conditioning (biofeedback) and behavioral habit training.

This year the Laboratory of Neurosciences successfully developed a tissue culture model that should prove extremely valuable for studies of brain plasticity. This laboratory has also adapted the Sokoloff (NIMH) technique to carry out sophisticated studies of aging in the brains of animals, including the use of pharmacologic probes to stress the system and identify any effects this might have on glucose metabolism rate in the brains of rats and dogs.

The same laboratory has successfully initiated clinical studies to examine regional brain metabolism in relatively healthy, normal subjects ranging in age from their 20's to 80's. PET scans are one tool being used by this group to study regional cerebral metabolic rates of glucose utilization.

Another group of LNS investigators have refined an osmotic method for opening the blood brain barrier and have quantified changes of cerebrovascular permeability in relation to infusate concentration and infusion time. They also demonstrated the reversibility of the osmotic method as a pharmacological tool. It was found that this method could be used in dogs to increase brain uptake of methotrexate, an anticancer agent, to the extent that it might be used to treat sensitive brain tumors. Collaborative studies with Dr. E. A. Neuwelt applied the osmotic procedure to man. As a result, it was shown that the blood-brain barrier in man can be opened without producing any apparent neurological changes. Now, a clinical Phase 1 study is underway to evaluate the osmotic method for use in treating unilateral brain tumors with methotrexate, or other anti-tumor agents.

Studies of the Baltimore Longitudinal Study of Aging cohort by clinical immunologists indicate that antibody levels to tetanus toxoid in humans decrease significantly with age. Such lowered antibody levels could make older adults more susceptible to tetanus than their younger peers. The female subjects studied seemed especially susceptible to tetanus bacteria as 60 percent of the women over 50 years of age had very low levels of anti-tetanus toxoid antibody.

In the Laboratory of Molecular Aging, Dr. Bruce Baum has found evidence disproving the generally held belief that taste sensitivity fades with age. A study of three age groups (20-39 years, 40-59 years, and 60-89 years) indicates that only 10 percent of healthy people experience loss of taste. On the other hand, 30 percent of those people on medication did report taste changes. The same investigator has shown that saliva flow does not change dramatically during the adult life span of healthy individuals. Investigation of 146 men and women in the BLSA cohort revealed that saliva production remains basically unchanged throughout life in healthy, non-medicated subjects. Here again, those on medication--especially postmenopausal women--did exhibit decreased saliva flow.

This year Laboratory of Molecular Aging scientists continued efforts to elucidate the function of vitamin D and its mechanisms of biochemical action. Even though Vitamin D_3 and its metabolites have been used to treat postmenopausal osteoporotic women, little is known about their function or the mechanisms of action. Using a recently established animal facility, LMA investigators produced Vitamin D deficient chicks to examine the effect of vitamin D status on the uptake of phosphate by kidney cells. In isolated cell preparations from these chicks, the researchers found the cells accumulated phosphate and a non-metabolizable sugar analog, alpha-methyl-D-glucoside, in response to a Na^+ gradient. They also found, for the first time, that in vivo administration of 1,25-dihydroxy Vitamin D_3 to rachitic chicks effected a 60% increase, within three hours, in the Na^+ gradient-dependent uptake of phosphate in the isolated cells.

Dr. Josef Pitha and co-investigators in the Laboratory of Cellular and Molecular Biology conducted studies of lipid soluble vitamins that are frequently used to treat age-related health conditions such as vitamin deficiency diseases. To be effective, however, large amounts of these vitamins, which can have toxic side effects, are required. One form of these vitamins, retinoids--derivatives of vitamin A--can reduce the incidence of lesions in animals treated with chemical cancer causing agents. The GRC scientists are trying to lessen the toxic effects of retinoids by transforming them into a water soluble form. This year, they synthesized and tested a new water soluble form of retinal which supports the growth of vitamin A-deficient, non-inbred rats as well as inhibits the proliferation of melanoma cells in culture. The new water soluble complex is less toxic than retinoids while retaining many of the beneficial effects. Development of this new complex has resulted in a successful patent application.

Sadly, the year included the loss of two outstanding investigators with the deaths, a few months apart, of Mr. Arthur H. Norris and Dr. Charles L. Goodrick. Mr. Norris, in addition to his scientific accomplishments, was the administrator of the BLSA for over 20 years and highly respected by our volunteers and his own staff in the Human Performance Section. Fortunately, we have obtained the services of Dr. Jordan Tobin to head the Section. Long an active research investigator at GRC, Dr. Tobin has had close working relationships with many of his new section's staff while a member of the Metabolism Section. The death of Charles L. Goodrick was a great loss to the field as he was the premier investigator in the world for the study of animal behavior and aging.

Office of the Scientific Director

Administratively, considerable progress took place this year in improving the physical facility and the surrounding grounds of the GRC. A new roof was installed, the air handling system is being upgraded, and landscaping has greatly enhanced the outward appearance of the building. All parking lots were resurfaced, relined, and mechanisms are being put in place to better control parking. The addition of curb cuts and reserved handicapped spaces to the front lot make the building accessible to disabled visitors, employees and longitudinal participants.

The appointment of a new Administrative Officer took place this year and has greatly facilitated operations. Support services for staff were bolstered by the addition of new computer systems, while library reference capabilities were enhanced. In the realm of employee interests, nearly all GRC supervisors received EEO training and a Center Awards Committee was established. Special efforts were made to publicize the Federal Incentive Awards program throughout the year. These efforts paid off handsomely for at least one staff member, Ms. Viola Beverly. She received the DHHS Secretary's Special Citation as one of this year's 10 departmental outstanding employees of the year. A laboratory worker in the Laboratory of Cellular and Molecular Biology, Ms. Beverly was the sole NIH employee winning this award in 1981.

Other staff cited or nominated included Mr. Wilbert Parsons, Experimental Morphology, and Mr. Maurice Zimmerman, Technical Development, who received a Presidential Citation for their development of a device to efficiently culture and harvest blood and splenic lymphocytes. Ms. Doris Hilferty, Library, was nominated for the 1981 NIH Merit Award, and Mr. Daniel Rogers, Information Office, received the NIH Recreation and Welfare Association Executive Council Award for service. Scientist Emeritus, Dr. Nathan W. Shock, received an Honorary Doctor of Humane Letters degree from Johns Hopkins University in recognition of his years of gerontological leadership.

The various units in the Office of the Scientific Director each made significant contributions this year as detailed in the following paragraphs.

Comparative Nutrition Section

Efforts have been made to establish whether dietary restriction increases life span by reducing the use of the genetic code and thereby minimizing genetic imperfections as they may occur in late life. It was decided to employ age-associated structural changes reported in aldolase purified from liver as the model to test this hypothesis. The use of this system depended upon whether these structural changes were due to transcriptional age-associated modifications. However, last year this laboratory reported that the age-associated changes in aldolase were post-translational. In addition, data accumulated since then indicated these changes were not consistent, i.e., they were present in only one out of four studies carried out. Therefore, it was felt advisable to reject this model system to test the hypothesis. Instead, efforts were made to establish two invertebrate systems which have been employed in studies concerning the relationship among dietary restriction, aging, and age-associated enzymatic structural changes. These systems are the rotifer and the nematode. Under our culturing conditions the life span of the rotifer provided unlimited access to food is twenty days and the egg production approximately forty-six eggs per rotifer. Experiments have shown that the life span of this organism can be increased from 19 to 28 days by dietarily restricting young-growing or adult organisms. These findings agree with those found in the current literature.

The culture conditions now employed yield concentrations of nematodes of approximately 150,000 to 200,000 organisms per ml. and also agree with data found in literature. During the coming year these new model systems will be used to test the hypothesis that dietary restriction increases life span by reducing use of the genetic code and thereby minimizing genetic imperfections as they occur in late life.

A positive relationship between the intake of specific vitamins and longevity has been suggested by such retrospective studies as that of Chope's of a population group in San Mateo, California, and Schlenker's study of another group in Michigan. In order to examine the relationship between vitamin intake and longevity in our laboratory, three groups of animals ages 30 days, 14 months, and 20 months were fed either vitamin amounts recommended by the National Academy of Sciences - National Research Council, one half this amount, four times this amount, or an amount of vitamins recommended by the American Institute of Nutrition. The results thus far indicate that feeding these amounts of vitamins to 20-month-old mice has no effect on longevity. Data is presently unavailable on the longevity of the younger group of animals. However, feeding a diet containing half the amount of the recommended vitamins resulted in markedly reduced body weight in both young-growing as well as the 14-month-old mice. There was no effect of vitamin intake on the body weights of the older animals.

The effect of dietary protein intake on hormone levels and the hormonal response to stress was determined in male Fischer 344 rats fed diets containing 4, 8, 12, 24, or 48% protein for 15 months. Plasma levels of corticosterone, aldosterone, testosterone, triiodothyrionine, thyroxine, and thyrotrophic stimulating hormone of non-stressed rats were elevated in immobilized rats fed the 48% protein diet to a greater degree than those found in animals fed the lower levels of protein. These findings appear to be a result of the increased need for conversion of protein to glucose in rats fed high-protein, low carbohydrate diets.

Experimental Morphology Section

This section serves as a Center-wide resource for scanning and transmission EM studies and conducts collaborative investigations with a number of laboratories within the NIA, other NIH components, and in university-based settings. As one example, Dr. Susie Humphreys is collaborating with Drs. Edward Lakatta and Arthur Kort of the Cardiovascular Section in a morphologic analysis of cardiac muscle. The EM section is looking at cardiac muscle fibers used in experiments where laser lights passes through the muscle. The electron microscope is used to determine fine structural changes in the muscle resulting from detergent treatments and which changes scattered light intensity fluctuations when the laser is used. Detergent "skinning" of muscle fibers stops scattered light intensity fluctuations. Interestingly, saponin dissolves most of the sarcolemma but not the sarcoplasmic reticulum, which is swollen in these fibers.

In other collaborative research the section is working with Drs. Uzi Reiss and Bertram Sacktor, Laboratory of Molecular Aging, to monitor regeneration of brush border in the proximal kidney tubule after isolateral ischemia. One hour of ischemia causes the kidney tubules to collapse and show degenerative changes. Microvilli swell, coalesce and lose parallel order. Other cytoplasmic changes include mitochondrial damage, cytoplasmic swelling and formation of large vesicles. The major differences noted to date between mature adult and aged rats is that morphologic recovery of brush borders after ischemia is slower and less common in the kidneys of older rats.

Animal Resources Facility

The Animal Resources Facility maintained its full accreditation, for the fourth consecutive year, with the American Association for Accreditation of Laboratory Animal Care. The Facility sustained its institutional membership with The National Capitol Area Branch of the American Association for Laboratory Animal Science (AALAS), and became an institutional member of the National AALAS.

Surgical and anesthesia skills and techniques were supplied by this section to aid in over 120 different research procedures including those on Rhesus monkeys, beagles, cats, and rodents. This past year marked the first time the Animal Resources Facility had a live Rhesus monkey born into the colony.

The efficiency of the facility was greatly improved with the addition of new rack washers, bottle washers, and a straight line tunnel washer. New mouse racks were placed in all experimental rooms, greatly increasing holding capacity. A new incinerator was installed.

Monthly inventories show that in the Open Animal Colony, care and housing were provided for 21,000 mice, 2,000 rats, 50 rabbits, 23 monkeys, 6 cats, and 200 chicks. Throughout the year, the facility received, maintained, or issued 11,000 mice, 6,100 rats, 700 rabbits, 15 dogs, 15 primates, 25 cats, and 5,300 chicks.

There are over 25,000 C57BL/6J mice in the aging mouse colony. Approximately 500 weanlings are added to the colony monthly. A total of 4,969 mice were issued from this colony; 3,264 were 1-23-months-old, and 1,705 were 24-months-old or older.

Aging Wistar Rat Colony is being maintained at 12,000 animals. There were 11,200 weanling rats introduced into the colony. A total of 3,158 rats were issued; 2,308 were 1-23-months-old,and 850 were 24-months-old or older.

Technical Development Section

The TechnicalDevelopment Section provided maintenance and development services to support the scientific programs of the Center. Several new instruments were developed and data processing facilities were substantially improved in 1981.

The VAX 11/780 computer system was installed in March of 1981, and 35 people have been trained and are using the new system. Presently, the system is being utilized at the rate of approximately 167 hours of connect time per week. All of the peripheral devices from the older systems have been interfaced and are functional on the new system. Software from the old systems has been converted to run on the VAX. Section staff are currently installing commonly used statistical packages such as BMD and SPSS. Also, several remote terminals in the building are connected to better use the system within the laboratory environment.

Every GRC program received support from the section's mechanical shops. Examples include: (1) Rotifer farms for the Comparative Nutrition Section; (2)

Gel slicers for the Inorganic Biochemistry Section; (3) Environmental chambers for Learning and Problem Solving Section; (4) Faraday cages for the Laboratory of Neurosciences; and, (5) 2 Stereo-Optic film mounts for the Experimental Morphology Section.

Electronic instruments were developed to meet the special needs of several GRC programs: (1) A liquid-chromatograph/fraction-collector microprocessor-based interface for the Laboratory of Molecular Aging; (2) Infrared motion detectors to collect data on mouse activity for the Learning and Problem Solving Section; (3) A liquid nitrogen level detector to assure an extended supply to the electron microscope in the Experimental Morphology Section; and, (4) A timer system to synchronize data collection from the PET Scanner for the Neuroscience Laboratory. Work continues on the development of a multiprocessor system to maintain several monkeys on operant conditioning schedules for the Psychophysiology Section.

Library

Even though the long empty position of Research Librarian remained vacant due to personnel restrictions, the GRC Library maintained quality services and advanced its capabilities this year. On-line reference services were strengthened by adding data bases in the Bibliographic Retrieval System and the NLM MEDLARS. Investigation by library personnel of ISI Original Tear Sheets (OATS) service solved the dilemma in locating hard-to-find domestic and foreign journals not available at the Library of Congress or NLM. An OCLC on-line system was installed in January 1981. This system is a realistic solution to the time consuming process of cataloguing books and preparing catalog cards.

The Library initiated a new policy for requisitioning books. The process developed by the staff, with procurement office approval, has improved the ordering process. Regular acquisitions of virtually all new pertinent titles are being received by date of publication. Over 700 new titles were added to the collection. A notable success this year was completion of classification and cataloguing of all books, both aging and research, as well as reference materials. Journals are now bound on a monthly basis with no backlog in journal binding.

During FY 1982, the Library will prepare a handbook describing its various services and policies as well as general library information. This will be distributed to all staff. This will greatly facilitate investigators and program staff obtaining needed documents in the future.

A 1200 baud computer terminal and printer will replace a portable 300 baud terminal, thus providing the technology needed to meet increased calls for reference services, and library serials and records control.

Information Office

GRC Information Office activities for the year culminated with a most successful Science Writers' Seminar, "Aging and the Behavioral Sciences," held June 3, 1981. The seminar, featuring all NIA intramural investigators, drew interest from some 60 media representatives with a third of these actually attending and others being sent full press kits at their request. Stories have appeared in the Baltimore Sun, another is due in Medical Tribune, and Newhouse Newspapers ran a three part series on aging, stimulated by the seminar exposure.

An arrangement was made with the Department of Family Practice, University of Maryland School of Medicine, whereby residents and interns enrolled in month long geriatric training, overseen by Dr. George Taler, will visit GRC as part of their activities. This cooperative program began in May 1981.

Public education efforts expanded with presentations given to a wide variety of audiences ranging from junior high school students and those from seven Baltimore/Washington colleges to members of the National Association of Retired Federal Employees, the Forum for Retired Professionals and Executives. Several outreach programs dealt with Federal job opportunities for Hispanic Americans, the handicapped, or for graduate students. Overall, 350 visitors took part in 54 briefings or tours of GRC.

Scripting was completed for a GRC orientation videotape with filming contingent on Photography and Arts staff availability in the summer of 1981. In connection with the upcoming White House Conference on Aging, the office set up successful reshooting of laboratories and scientists to update the NIA's videotape, "Understanding Aging Through Research." In addition, permission has been obtained to use CBS-TV's excellent 1980 magazine segment on the BLSA for the Institute's conference exhibit. Also, consideration is being given to providing certain testing equipment for demonstration at the meeting.

Information staff took on the monumental task of preparing the manuscripts for the 1977 exercise conference and BLSA books being prepared by the Institute. For the exercise conference, by June 1981, all chapters had been processed at least for the first time. Introductory and several other chapters have been partially processed for the BLSA book.

Over the year, the Information Office received or initiated 176 media contacts. A number of stories appeared on national, regional, and local television and radio stations as a result. Plans are also in process to have the "NOVA" science series, WCVB-TV (Boston), and Omni TV Productions film in Baltimore this fall. Print media stories appeared in The Washington Post (2), Baltimore Morning Sun (2), Baltimore Evening Sun, Staten Island (N.Y.) Advance, McCall's, New Scientist, Psychology Today, Medical Tribune, Archives of Internal Medicine, JAMA, Self Magazine, Prevention, Next Magazine, Catonsville (Md.) Times, and Arbutus (Md.) Times.

Preparations have just begun to plan and hold a gala celebration to herald the 25th anniversary of the BLSA, scheduled to occur in 1983. A planning committee is in place and held its first organizational meeting this summer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00101-05-SCN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Relation between Nutritional State and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. H. Barrows	Chief	SCN, OSD, NIA
Other:	G. C. Kokkonen	Chemist	SCN, OSD, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Office of the Scientific Director

SECTION

Section on Comparative Nutrition

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data accumulated during this current year indicated that the age-associated structural changes reported last year in aldolase of liver were not consistent, i.e., they were present in only one out of four studies carried out. Therefore, it was felt advisable to reject this model system to test the hypothesis that dietary restriction increases life span by reducing the use of the genetic code and thereby minimizing genetic imperfections as they may occur in late life. Instead, efforts have been made during the past year to establish two invertebrate systems which have been employed in studies concerning the relationship among dietary restriction, aging, and age-associated enzymatic structural changes. These systems are the rotifer and the nematode. Under our culturing conditions the life span and egg production of the rotifer, as well as the population density of the nematode, are in agreement with those found in the current literature. Three groups of animals ages 30 days, 14 months, and 20 months were fed varying amounts of vitamins. The results thus far indicate that feeding these amounts of vitamins to 20 month old mice has no effect on longevity. No data is presently available on the longevity of the younger groups of animals.

Objectives: Attempts were made to establish whether selective errors occur in the genetic code as a function of age and/or use and whether such changes are delayed by dietary restriction. In addition efforts were made to determine the effect of vitamin intake on life span.

Methods Employed: In four experiments the activity of aldolase and the concentration of protein were determined in cytosols isolated from the livers of 7.5 (N=65) and 30 month old (N=74) C57BL/6 female mice fed Purina Laboratory Chow and obtained from the Gerontology Research Center and Charles River animal facilities. In addition, aldolase from the livers of the animals was purified by ion-exchange chromatography on a phosphocellulose column using a linear salt gradient followed by substrate elution and the specific activity of the purified preparations determined.

Three groups of C57BL/6J male mice, ages 30 days, 14 months, and 20 months were fed *ad libitum* diets containing either an amount of vitamins recommended by the National Academy of Sciences - National Research Council, one half this amount, four times this amount, or an amount of vitamins recommended by the American Institute of Nutrition. Each dietary age group contained 50 animals. The body weights and life span of these animals were determined.

Axenic cultures of *Turbatrix aceti* were obtained from Dr. Krusberg at the University of Maryland. The organisms were grown in 1000 ml. polystrene culture flasks containing 50 ml. of a basal defined medium with or without 10% heated liver extract. The basal defined medium contained all the amino acids and known vitamins. In addition, two sterols were added: 25 ug./ml. of cholesterol and 25 ug./ml. of B-sitosterol. Hemoglobin was added to a final concentration of 500 ug./ml. after first being dissolved in a minimal volume of water. The ph of the medium was adjusted to 5.8 with potassium hydroxide and acetic acid was then added to a final concentration of 4 percent by volume. These cultures were subcultured routinely every 2-3 weeks by transferring worms to provide a concentration of approximately 10,000 organisms/ml. in the new culture. Cultures were incubated at 30°C.

Rotifers, obtained from the Carolina Biological Co., were cultured in a medium containing varying concentrations of *Enterobacter aerogenes* previously cultured in nutrient broth and appropriately diluted with a phosphate buffer containing calcium and magnesium ions. Stock cultures were grown in 15 ml. of medium containing approximately 35×10^6 bacteria/ml. in 25 ml. capacity glass culture dishes and changed three times per week. During the experiments, rotifers were isolated in glass depression plates and maintained in 0.5 ml of medium. Isolated rotifers were changed to fresh medium every weekday during their egg-laying period and every other day thereafter until death. All organisms were incubated at 30°C.

Major Findings: In four experiments there was a statistically significant ($p<.001$) age-associated decrease in the specific activities of the isolated cytosols. This decrement was the result of a decrease in the enzymatic activities rather than an increase in the protein contents of the cytosols of livers of old animals. However, in only one experiment of the four was there also an age-associated decrease in the specific activities of the purified preparations. In this experiment the decrease in the specific activity of the cytosol was approximately 40% whereas in the other experiments it was approximately 20%. Examinations of the liver and body weights or the protein contents of the cytosols indicated no age changes which might have accounted for the age decrement in the specific activity of this purified preparation.

The results thus far indicate that vitamin intake has no effect on the longevity of 20 month old mice. No data is presently available on the longevity of the younger groups of animals. However, feeding a diet containing half the amount of the recommended vitamins resulted in markedly reduced body weights in both the young-growing as well as the 14 month old mice. There was no effect of vitamin intake on the body weights of the older animals.

Thus far the two invertebrate systems have been established to the following extent. Under our culturing conditions the life span of the rotifer under normal nutritional conditions is twenty days and the egg production has been found to be forty-six eggs per rotifer. These results are in agreement with that found in the literature. In addition, experiments have shown that the life span of this organism can be increased from nineteen to twenty-eight days by dietarily restricting young-growing or adult organisms. The culture conditions employed in this laboratory yield population densities of nematodes of approximately 150,000 to 200,000 organisms per ml. and is in agreement with data found in the literature.

Significance to Biomedical Research and the Program of the Institute: Reports of experiments attempting to determine the age-associated presence of inactive aldolase in the livers of rats and mice have been found in the literature since 1973. The findings to date have been inconsistent. Variations in biochemical methods, bases for expressing enzymatic activities, strain, source and age of animals used have made an assessment of these inconsistencies difficult. In order to finally obtain a definitive answer to this long-standing question this laboratory employed the same biochemical techniques on both crude and pure aldolase preparations from C57BL/6 female mice obtained from two sources. The one consistent finding in this report is an age-associated decrease in the specific activity of liver cytosols, a suggestion that a reduced synthesis of the enzyme occurs with age. The inconsistency in the presence of altered enzyme indicates that the formation of inactive aldolase may occur in some old animals but apparently is not due to aging *per se*.

Recent retrospective studies suggest a beneficial effect could result from consuming vitamins in quantities greater than the recommended daily allowances. Chope's data suggests a relationship exists between mortality and the intakes of vitamins A, C, and niacin. For example, among individuals consuming less than 5,000 IU of vitamin A per day, a mortality of 13.9% was observed. For those subjects whose intake was above 5,000 IU per day, the mortality was 5.4%. Similar data for vitamin C was obtained by Schlenker. However, on the basis of the low frequencies of nutritional deficiencies and the complete lack of information on the effect of continued long-term vitamin therapy in older people, it is unwise to propose mass vitamin and other nutrient supplementation to the aged at this time. The vitamin experiments undertaken by this laboratory when concluded will provide valuable data which can be used to determine guidelines to be followed for long-term vitamin supplementation.

Proposed Course:

1) To continue experiments relating vitamin intake to longevit in the mouse.
2) To establish the relationship among dietary restriction, aging, and age-associated enzymatic structural changes in the rotifer and nematode.

Publications:

Barrows, C. H. and Kokkonen, G. C.: The Effect of Age on Aldolase in the Livers of Rats. *Age* 3: 53-58, 1980.

Barrows, C. H. and Kokkonen, G. C.: *Comparative Nutrition during Aging*. In Rechicgl, M. (Ed.): Comparative Animal Nutrition, vol. 4 Physiology of Growth and Nutrition, Basel, Karger Press, in press.

Barrows, C. H. and Kokkonen, G. C.: Research Needs. In Hsu, J. M. and Davis, R. L. (Eds.): *Handbook for Nutrition and Diet of Aging: Principles and Applied Aspects*, Park Ridge, N. J., Noyes Publications, in press.

Barrows, C. H. and Kokkonen, G. C.: The Effect of Induction of Aldolase on the Livers of Senescent Mice. *Age* 4: 1-3, 1981.

Barrows, C. H.: Centenarians. In Hopps, H. C. (Ed.): *Aging and the Geochemical Environment*, Wash., D. C., National Academy Press, in press.

Barrows, C. H.: The Effect of Age on Physiological Functions. In Hopps, H. C. (Ed.): *Aging and the Geochemical Environment*, Wash. D. C., National Academy Press, in press.

Barrows, C. H. and Kokkonen, G. C.: Dietary Restriction and Life Extension - Biological Mechanisms. In Moment, G. B. (Ed.): *Nutritional Approaches to Aging Research*, Boca Raton, CRC Press, in press.

Barrows, C. H. and Kokkonen, G. C.: Genetic Information and the Life-Span of Laboratory Animal Models. In Segal, M. (Ed.): *Reviews in Pure and Applied Pharmacological Sciences*, London, Freund Publishing House, and Chichester, John Wiley & Sons, vol. 1, 293-325, 1980.

Barrows, C. H. and Kokkonen, G. C.: Inconsistencies in the Presence of Age-Associated Altered Aldolase in the Livers of Mice. *Age*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00141-04-SCN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effect of Aging on Vitamin Absorption

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Fleming	Staff Fellow	SCN, OSD, NIA
	C. H. Barrows	Chief	SCN, OSD, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Office of the Scientific Director

SECTION

Section on Comparative Nutrition

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project was to examine the effect of age on absorption of fat-soluble and water-soluble vitamins. Female Wistar rats, aged 6, 12, and 24 months were orally administered radioactive forms of vitamins A and D were sacrificed 18 hours later. A second group of 6, 12, and 24 month old rats were also orally administered radioactive B_{12} and niacin and sacrificed 16 hours later. In both experiments, the esophagus, stomach, small intestinal wall, contents of the small intestine, cecum, colon, and collected feces were assayed for isotope. Liver and plasma levels of the administered vitamins were also determined at sacrifice. Absorption of each of the vitamins was unaffected by age. Plasma and liver levels of the isotope at sacrifice were also not related to the age of the animals.

This project has been discontinued.

GRC/OSD-12

PHS-6040
(Rev. 2-81)

Project Description Z01-AG-00141-04-SCN

This project has been discontinued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00143-02-SCN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effect of Protein Intake on Serum Hormones, Creatinine, and Cholesterol

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Fleming	Staff Fellow	SCN, OSD, NIA
Other:	C. H. Barrows	Chief	SCN, OSD, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Gerontology Research Center, Office of the Scientific Director

SECTION
Section on Comparative Nutrition

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of the present study was to examine the effect of dietary protein intake on hormone levels and the hormonal response to stress. Male, Fischer 344 rats were fed diets containing 4, 8, 12, 24, or 48% protein for a 15 month period. Plasma levels of corticosterone (CS), aldosterone, testosterone, triiodothyronine (T3), thyroxine (T4), thyrotrophic stimulating hormone (TSH), total protein, and albumin were determined at several ages in non-stressed rats and in rats stressed by immobilization. Hormone levels of non-stressed rats were not influenced by dietary protein intake. Stress resulted in significantly increased levels of corticosterone and aldosterone while T3, T4, and TSH were not elevated as a result of immobilization. Corticosterone and aldosterone levels were elevated in immobilized rats fed the 48% protein diets in comparison to the 4, 8, 12, and 24% groups. By 3 hours following immobilization, CS and aldosterone levels in the 48% group were similar to prestress levels and similar to the other dietary groups. The apparent hyper-responsiveness of the adrenal of high protein fed rats may be a result of the increased need for conversion of protein to glucose in rats fed high protein, low carbohydrate diets.

Objectives: The objective of the present study was to examine the effect of dietary protein intake on hormone levels and on the hormonal response to stress in rats. A preliminary study of 14 month old female Fischer 344 rats resulted in findings of increased triiodothyronine (t3) and thyroxine (t4) levels and decreased levels of aldosterone and corticosterone in serum of rats fed 4 percent protein while the levels of these hormones did not differ among rats fed 8, 12, and 24% protein. Nor were TSH, prolactin, creatinine, triglyceride, or cholesterol levels in serum affected by dietary protein intake in the female Fischer 344 rats. The present study was undertaken to follow-up and extend the study with the female Fischer 344 rats using male Fischer 344 rats fed 4, 8, 12, 24, or 48% protein.

Methods Employed: Male Fischer 344 rats were obtained at weanling, housed singly, and fed diets containing 4, 8, 12, 24, and 48% protein during the 15 month period of the study. At various times during this period, serum samples were taken from unstressed animals and assayed for t3, t4, corticosterone, aldosterone, and testosterone. Total serum protein and albumin levels were determined in the rats at 15 months of age.

Prestress levels of hormones were determined in a group of rats not subjected to stress. At 3 different ages, groups of rats were immobilized and their hormonal response to stress was determined. The procedure used for producing stress consisted of placing the rats in a plastic holding device for 20 minutes. Hormone levels were determined immediately after the rats release from the device. The 3 hour post-stress hormone levels were determined in a third group of rats who were immobilized and then returned to their home cages for 3 hours prior to sampling.

Prestress levels of corticosterone (CS) determined in the rats at 3, 11, and 15 months of age did not differ among the 5 dietary groups. Highly significant increases in CS levels occurred in all dietary groups as a result of immobilization. Among stressed rats, CS were highest in rats fed the 48% diet. By 3 hours after immobilization, CS levels had returned to approximately pre-stress levels and did not differ among the dietary groups.

Prestress or baseline levels of hormones assayed did not differ as a result of dietary protein intake in these rats at the ages examined. Stress resulted in significantly higher levels of CS and aldosterone in rats fed 48% protein diets.

Testosterone levels will be determined in serum collected at sacrifice of the 15 month old rats. Plasma total protein and albumin will also be determined in this group.

A preliminary study conducted in female rats (reported in the 1980 annual report) suggested that increased levels of thyroid hormones and decreased levels of adrenal hormones occurred in rats fed 4% protein. The present study with male rats did not confirm the finding of differences in hormone levels of non-stressed rats. However, the adrenal hormones were elevated in rats fed the 48% protein diets in the present study. The mechanism and significance of this result is not known. Increased levels of CS would result in increased production of glucose from protein which may be essential in rats fed an inadequate amount of dietary carbohydrate such as is the case with rats fed 48% protein. Increased aldosterone output would then result from the increased ACTH necessary to produce increased stimulation of gluconeogenesis via the corticosteroids.

Major Findings: Rats fed the 4% protein diet were significantly smaller than rats fed the 8, 12, and 24% protein diets after 4 weeks of consumption of the diets and remained smaller throughout the study. Rats fed the 48% diet did not gain weight as rapidly at the 8, 12, or 24% groups, a trend which was particularly evident after about 6 to 7 months consumption of the diet.

Feed efficiency was less in the 4% protein group compared to the other groups as indicated by consumption of more food per 100 g of body weight in this group. This difference in feed efficiency among the groups was less noticeable as the rats aged.

The thyroid hormones and thyrotrophic stimulating hormone levels in serum were assayed in a representative group of rats from each dietary protein group. At 5 months of age, prestressed, stressed, and post-stress levels of t3, t4, and TSH were determined. Stress did not result in a significant increase in any of these hormones in any dietary group. The non-stressed rats fed the 4% protein diet did have elevated levels of t3 at 5 months of age, although this difference was not evident at 14 months. There were no differences among the other diet groups in levels of t3, t4, or TSH at 5 or 14 months of age.

Aldosterone levels were determined for prestress, stress, and 3 hour post-stress periods in the rats at 11 months of age. Prestress levels of aldosterone did not differ among the dietary groups. Aldosterone levels were significantly greater in stressed rats in all 5 dietary groups with the greatest percent increase compared to prestress levels occurring in rats fed the 24 and 48% diets. Aldosterone levels were increased 179, 208, 251, 406, and 358% in stressed vs prestressed rats in the 4, 8, 12, 24 and 48% groups.

Significance to Biomedical Research and Program for the Institute: Examination of the effect of different levels of dietary protein on plasma hormone levels and the plasma hormone response to stress is of importance from both a practical and a theoretical point of view. The wide range of protein intake consumed by individuals throughout the world makes evaluation of protein intake and its influence on hormonal status important. The role of protein intake as a modulating factor in the organism's ability to respond to stress is also of practical importance.

A decrease in the level of both protein and total calories in the diet has been reported to result in increased life span. Although this finding has been more extensively documented in the case of caloric restriction, several investigators have reported an increased life span in protein restricted animals. Arthur Everitt has proposed that dietary restriction is equivalent to hypophysectomy and that this mechanism is responsible for the lengthened life observed in restricted animals. The present study does not support the theory that restriction is equivalent to hypophysectomy and thus suggests that this mechanism (hypophysectomy) is not responsible for the increased life span observed by several investigators in protein restricted rats.

Proposed Course of Study: Plasma testosterone levels and plasma protein and albumin levels remain to be determined. After determination of these factors, the study of the effect of protein intake on plasma hormone levels and on the stress of response will be concluded.

Publications:

Fleming, B. B.: (in press) The vitamin status and requirements of the elderly. In: Nutritional approaches to aging research. (ed.) G. B. Moment, CRC Press, Boca Raton, Florida.

Fleming, B. B., and C. H. Barrows: (in press) The influence of aging on intestinal absorption of vitamin B_{12} and niacin in rats. Exp. Geront.

Fleming, B. B., and C. H. Barrows: (in press) The influence of aging on intestinal absorption of vitamins A and D by the rat. Exp. Geront.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00102-05-SCN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Comparative Mammalian Biochemistry
I. Stability of Gene Control Mechanism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

R.G. Cutler	Research Chemist	SCN, NIA

COOPERATING UNITS (if any)
none

LAB/BRANCH
Gerontology Research Center, Office of the Scientific Director

SECTION
Section on Comparative Nutrition

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0.33	PROFESSIONAL: 0.33	OTHER:

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work has continued on determining if (1) there is an age-dependent loss at the cellular level of proper gene regulation and (2) whether there is a correlation between a mammalian species' innate longevity or aging rate and the intrinsic ability of cells to maintain their proper state of differentiation. Studies on the age-dependent expression of murine leukemia virus in thymus, brain and liver of AKR mice have been completed. Results indicate that even non-target tissues of leukemia virus, such as liver, show an age-dependent increase in the amount of leukemia-like RNA in the nuclei. We have also found a good correlation between a mammalian species' innate aging rate and its age-dependent onset frequency of various types of cancer. Such data suggests that cells of longer-lived species may have a life-long higher stability against genetic modifications leading to cancer. Thus, cancer and aging may have both common causes and rate-governing processes.

PHS-6040
(Rev. 2-81)

Project Description:

Objective: Medical research in the past has predominantly been involved with the type of human pathology which is caused by and/or originated from external environmental influences. Very little is known about the extent and biological nature of human pathology of an intrinsic origin--that is, the pathology of an endogenous nature which originates from within an organism. Even less is known of how these dysfunctions might best be treated. The age-dependent dysfunctions collectively called "aging processes" probably represent the most important potential class of intrinsic pathologies. It is the general objective of our research program to contribute towards our understanding of the biological nature of these intrinsic types of dysfunctions and in particular to determine what natural counter-processes may exist to protect an organism.

The working hypothesis I have used in guiding our research efforts predicts (1) that the origins of intrinsic dysfunctions are pleiotropic in nature and are the normal products and by-products of metabolism and development, (2) these pleiotropic products are predicted to alter the proper differentiated state of cells in an epigenetic/mutational manner, which then leads by a cascading effect to the syndromes of aging, and (3) specific longevity determinant processes exist which govern the period good health is maintained. These longevity determinant processes determine the time-dependent stability of the differentiated state of cells throughout an organism--and thus its longevity.

Our general research approach has been divided into three related areas of investigation: (1) stability of gene control mechanisms, (2) characterization of chromatin, and (3) levels of repair and protective processes. This report covers the first area of investigation, where work has continued in determining if: (1) there is an age-dependent loss at the cellular level in proper gene regulation and (2) whether there is a correlation between a mammalian species' longevity and the ability of cells to maintain their proper state(s) of differentiation in the presence of known toxic and mutagenic/carcinogenic agents.

Methods Employed: The general method used to investigate improper specific gene expression is to search for the presence of RNA transcribed by a gene in cells where this gene would not normally be expected to be expressed. The RNA is detected by using a radioactive complementary DNA (cDNA) probe. The cDNA probe is obtained by isolating pure messenger RNA known to code for a specific gene product and then using this RNA as a template with reverse transcriptase enzyme to synthesize the radioactive cDNA probe. RNA is then extracted from the nuclei and/or cytoplasmic fractions of certain tissues and the presence of RNA complementary to the cDNA probe is determined by DNA·RNA hybridization techniques.

The correlation found between mammalian species' aging rate and age-dependent onset frequency of various types of cancer was a result of compiling data obtained from published papers.

Major Findings: We have now completed the studies we initiated five years ago on examining the possible age-dependent increase of specific genes in cells where their expression would not be expected. These genes are alpha- and beta-globins, casein, alpha-fetoprotein, the set of genes making up the endogenous mouse leukemia virus (MuLV), and the set of genes making up the endogenous mouse mammary tumor virus (MMTV).

The results with mouse indicate that the endogenous viruses studied are indeed expressed to a greater extent qualitatively as a function of age in non-target tissues. Whether this is only a characteristic of mouse which would not be found in primates (using equivalent primate endogenous viruses) remains to be determined and represents an important extension of these experiments.

Moreover, our results could be characteristic of only highly inbred mouse strains, so similar experiments using wild-type outbred mouse strains should also be undertaken. We have also found an age-dependent increase in the expression of globin and alpha-fetoprotein in human tissues, but these studies have not been completed to the necessary extent to make these findings truly significant. The only structural genes that clearly increase with age are alpha- and beta-globin in mouse brain. The casein gene did not show any age-dependent change. Thus, the globin results may be a special case, and more structural genes where the cDNAs are readily available, such as growth hormone, insulin and albumin, need to be studies. Our most recent studies using the short-lived AKR mouse strain, which has a high incidence of leukemia, have shown that the age-dependent derepression of globin and mouse leukemia RNA in brain and liver is not necessarily linked. Thus, genetic factors can alter the pattern of gene derepression, much like different tissues show characteristic patterns of aging.

Few investigators in the field of cancer have seriously considered the possibility that the processes determining the well-known age-dependent onset frequency of many different types of cancer may be the same processes as those determining the age-dependent onset frequency of non-cancerous dysfunctions. It is generally agreed that the age-dependent increase in onset frequency of cancer is considered to be only dependent on chronological time and not physiological age. For example, for a given mammalian species, experiments have indicated that physiologically increased age does not appear to result in an increased sensitivity to carcinogenic agents. We have predicted, however, that aging and cancer are linked in a causative and rate-determining manner, where (1) both aging and cancer are a result of an accumulation of steps proceeding in a time-dependent manner and (2) the rate of how fast these steps proceed is determined by the levels of intrinsic longevity determinant processes that are species-specific; the rate is therefore primarily a function of innate longevity potential and not chronological age.

In support of these predictions, we have found (1) a linear positive correlation of tissue levels of superoxide dismutase per unit specific metabolic rate as a function of primate maximum lifespan potential and (2) a negative correlation between the maximum lifespan potential of primates and the onset frequency of a number of different types of cancer. Such data suggests that the cells of longer-lived species have an inherently greater life-long stability against genetic modifications leading to dysfunctions, including cancer as well as aging. Thus, most types of cancer may represent a special class of dysdifferentiated cells, the most common being those that accumulate with increasing age and which have only a slight decrease in normal function. It is this latter class of cells which results in the dysfunctions associated with aging.

Significance to Biomedical Research and Program of the Institute: These findings so far indicate that all structural genes do not undergo an age-dependent derepression and that, instead, this derepression is likely to be highly specific, not only to the type of structural gene involved but also to the type of tissue, cell fraction (nuclei or cytoplasmic) and strain or species of animal. In contrast to structural genes, however, an age-dependent derepression of endogenous c-type viruses related to cancer and other diseases may be universal. These results, if confirmed by more extensive studies which are underway, could lead to the identification of an important genetic alteration of cells which may underlie many of the mammalian aging processes.

Proposed Course of the Project: Our proposed course is to determine (1) the generality of age-dependent relaxed gene expression in terms of tissues and number of different genes affected, (2) if a correlation exists between aging rate of a species and the rate of loss of proper gene control, (3) the molecular mechanisms leading to a loss of gene control and (4) to identify the biological processes that may govern the age-dependent stability of gene expression. These latter two objectives are the projects of the second and third parts of our research program.

Publications:

Cutler, R.G.: Evolution and genetics of human longevity. to appear in: Viidik, A. (ed.) Lectures on Gerontology, Academic Press, London.

Cutler, R.G.: Lifespan extension. in press: McGaugh, J. (ed.) Biology, Behavior, and Aging, Natl. Acad. Sci., Washington, D.C.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00103-05-SCN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Comparative Mammalian Biochemistry
II. Characterization of Chromatin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

R.G. Cutler	Research Chemist	SCN, NIA

COOPERATING UNITS (if any)

Laboratory of Nutrition and Endocrinology, NIAMD

LAB/BRANCH
Gerontology Research Center, Office of the Scientific Director

SECTION
Section on Comparative Nutrition

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0.33	PROFESSIONAL: 0.33	OTHER:

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Age-dependent changes in the differentiated state of cells would be supported if evidence could be found for physico-chemical changes occurring in the chromatin. In addition, if cells of longer-lived mammalian species are inherently more stable in maintaining their proper state of differentiation as a function of time, then this characteristic may be reflected in some special or unique physico-chemical properties of the chromatin. To further examine these two possibilities, we have been measuring the ribosomal RNA gene dosage in mouse and primate species as a function of age and of maximum lifespan potential. Such changes in ribosomal RNA gene dosage appear probable in view of the apparent dynamic state of DNA and the presence of transposed elements (TE's), which could not only affect gene dosage number but also the chromosomal arrangement of genes. These changes could in principle alter the differentiated state of cells. Results to date indicate no age-dependent change in ribosomal RNA gene dosage in man or mouse, but more extensive and sensitive tests are being planned.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: This project is designed to investigate whether the genetic apparatus of cells remains intact throughout the lifespan of the animal or if significant physico-chemical or mutational alterations occur that could lead to the improper function of the cell and possibly to the aging of the organism. The specific objective of these studies is to search for alterations at the chromatin level which might help explain the possible dedifferentiation processes that have been found to occur with increased age in mouse brain and liver tissues.

It has occurred to us that, if cells from longer-lived species are indeed inherently more stable in their ability to maintain their proper state of differentiation, then this characteristic might be reflected in some type of unique physico-chemical differences in the chromatin. For example, it has been reported that an age-dependent loss of rRNA genes occurs in human tissues. A possible mechanism involving such a gene loss could be related to the recently recognized dynamic state of DNA (such as with the transposable elements), where migration of DNA segments might be occurring even after the differentiation and developmental program of a cell has been completed.

Our earlier investigations into possible age-dependent loss of rRNA genes in mouse and human have showed no age-dependent change with age. However, because of new techniques now available, these studies should be repeated. In addition, we plan to determine if ribosomal gene dosage is related to a primate species' maximum lifespan potential.

Recent advances in the characterization of chromatin proteins by polyacrylamide two-dimensional gel electrophoresis will be used to determine if unique differences exist in the types and amounts of chromatin proteins in these primate species which can be correlated with their maximum lifespan potentials.

Methods Employed: A liquid DNA·rRNA hybridization technique will be used with radioactive rRNA obtained from human tissue culture cells. Both 18s and 28s rRNA types will be utilized in a hybridization process using the single-stranded specific enzyme S_1 nuclease.

Major Findings: Our findings to date have been limited to mouse and human, and so far we have found no age-dependent changes. Results for the non-human primate species have not been completed. Species comparison of chromatin using the two-dimensional polyacrylamide gel electrophoresis technique have also not yet been completed.

Significance to Biomedical Research and Program of the Institute: Much of the aging process could theoretically be explained by an age-dependent alteration of the genetic apparatus of cells. It is therefore essential to determine if chromatin (the major genetic constituent of cells) is altered with age, and if so, if this alteration is reversible. The age-dependent and the longevity-dependent stability of redundant classes of genes such as the rRNA genes is important to establish, in view that such a gene loss could reflect more extensive alterations occurring in the arrangement of genes within a cell. Such knowledge is considered essential to determine the biological nature of aging. Moreover, a knowledge of the processes governing the duration good health is maintained, such as the possibility of unique chromatin proteins, could conceivably lead to the development of means to enhance the normal expression of such health maintenance processes.

Proposed Course of Project: We plan to complete the rRNA gene studies on human and non-human primates and to undertake a comparative structural determination of chromatin composition in primate species as a function of their MLP using a two-dimensional polyacrylamide gel electrophoresis technique.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00105-04-SCN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Comparative Mammalian Biochemistry
III. Levels of Repair/Protective Processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

R.G. Cutler	Research Chemist	SCN, NIA

COOPERATING UNITS (if any)
David Harrison, Jackson Laboratory, Bar Harbor, Maine
Donald Ingram, GRC, NIA

LAB/BRANCH
Gerontology Research Center, OFfice of the Scientific Director

SECTION
Section on Comparative Nutrition

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.33	0.33	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have continued on determining the processes governing tissue levels of superoxide dismutase (SOD) and other potential longevity determinant processes. We have completed the project investigating the role the major histocompatibility complex may play in determining tissue levels of SOD. Cu/Zn and Mn types of SOD were measured in the liver of 10 different H-2 congenic strain mice. No significant correlation was found with H-2 type nor with their lifespan. We have also completed studies determining the SOD levels in liver of parabiosed mice of different H-2 congenic mouse strains to observe possible effects of humoral factors on SOD tissue levels. No effect is evident. Finally, SOD levels in liver of rats have been determined that had been exercised voluntarily throughout their lifespan. Results show no enhancement of SOD levels as compared to non-exercised controls.

Project Description:

Objectives: This project represents a search for genetic repair and protective processes which might act to govern the time-dependent stability of gene expression, and particularly those processes which may play an important role in general maintenance of health in man. The approach used is to compare levels of potential genetic repair and protective processes with the maximum lifespan potentials of different mammalian species, with emphasis being placed on the primate species. Recent studies have concentrated on determining what genetic and environmental factors might influence tissue levels of SOD.

Methods Employed: The pyrogallol and xanthine/xanthine oxidase methds for determining SOD tissue levels were used in all studies. Cyanide inactivation of the Cu/Zn SOD type was used to distinguish between the Cu/Zn and Mn types of SOD. Parabiosed H-2 congenic mouse pairs were prepared by Dr. David Harrison of the Bar Harbor Laboratory, Bar Harbor, Maine. Volunteer-exercised and non-exercised rats were furnished by Dr. Don Ingram of the GRC.

Major Findings: It was reported by another laboratory (Novak *et al.*, *Science* 207:86-87, 1980) that SOD levels in liver of mice appear to be influenced by allelic changes occurring only within the major histocompatibility loci. We undertook to determine if this effect could be repeated in our laboratory. The importance of such a finding, if true, would suggest that genes governing tissue levels of SOD as well as other possible longevity determinant processes may reside in the MHC. It has already been proposed that DNA repair and maximum lifespan potential are influenced by allelic changes occurring only within the MHC.

Ten different H-2 congenic strain mice were used that were the same strains used in the previously reported studies on SOD and where lifespan differences have been recorded. The SOD levels were determined in crude as well as purified preparations from liver. Both Cu/Zn and Mn SOD types were assayed. Results indicate no significant H-2 correlation of SOD levels in liver (see table for summary of results).

The experiments conducted to determine if H-2 differences between mouse strains might influence endogenous tissue SOD levels through a humoral factor were also negative. Parabiosed mouse pairs were used, consisting of a number of different H-2 congenic strain types. No differences in the parabiosed mice were noted after they had been together for about six weeks, as compared to non-parabiosed mice.

If superoxide dismutase is an important protective enzyme against oxygen metabolism by-products, then increased exercise might be expected to induce SOD to higher levels. In support of this prediction is the observation that rats undergoing voluntary exercise throughout their lifespan have a higher basal metabolic rate by about 25-30 % as compared to non-exercised rats. However, on comparing the levels of Cu/Zn and Mn SODs in liver, no significant differences were evident between exercised and non-exercised animals.

Significance to Biomedical Research and Program of the Institute: These studies do not support the concept that SOD and perhaps other potential longevity determinant processes are governed by genes residing near or within the major histocompatibility complex. However, more experiments need to be conducted, particularly with other enzymes such as those involved in DNA repair and purine detoxification processes to evaluate the important concept that a common set of genes within the MHC may govern both the functions of the immune system and aging rate.

SUPEROXIDE DISMUTASE LEVELS IN LIVER OF H-2 CONGENIC MOUSE STRAINS[1]

MOUSE STRAIN	H-2 ALLELE	10th DECILE LIFESPAN (WKS)	WHOLE CELL (CRUDE) UNITS/MG PROTEIN		CYTOSOL (CRUDE) UNITS/MG PROTEIN		CYTOSOL (PURIFIED) UNITS/MG PROTEIN	
			Cu/Zn[2]	Mn	Cu/Zn	Mn	Cu/Zn	Mn
C57Bl/10Sn	b	155	24.2 (46)	37.9	33.5	16.8	1290	42.7
B10.A/SgSn	a	154	20.0 (15)	16.3	22.3	22.3	1336	47.1
B10A(2R)/SgSn	h_2	-	32.8 (46)	21.8	23.4	19.4	-	-
B10A(5R)/SgSn	i_5	-	31.1 (18)	13.5	30.1	18.1	-	-
C3H.SW/Sn	b	150	21.5 (58)	13.1	29.5	43.0	1860	45.0
C3H/HeSn	k	138	29.0 (18)	13.5	20.9	17.1	1653	46.2
B10.AKM/Sn	m	139	34.4 (23)	3.46	36.6	3.89	1495	62.3
B10.D2/nSn	d	157	10.2 (15)	16.9	20.3	13.4	1508	49.9
A.BY/Sn	b	114	35.1 (45)	10.0	18.7	16.2	1003	37.8
B10.BR/SgSn	k	149	29.2 (23)	20.6	32.1	10.5	1102	26.6

[1] Superoxide dismutase determined by the xanthine/xanthine oxidase assay.

[2] Values in parenthesis for Cu/Zn are reported by Novak *et al.*, *Science* 207:86-87, 1980.

The above results have failed to confirm any correlation of SOD levels in tissues of mice as a function of their H-2 allele type or lifespan. Similar results were found for brain and spleen.

Proposed Course of the Project: Studies are now underway on determining tissue levels of both Cu/Zn and Mn SODs in primate species as a function of their MLP, as well as levels of glutathione peroxidase and glutathione.

Publications:

Cutler, R.G.: Lifespan extension. in press: McGaugh, J. (ed.) Biology, Behavior, and Aging, Natl. Acad. Sci., Washington, D.C.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00151-02-OSD

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Aggregation Factor Visualized on Aggregating Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	EMS, OSD, NIA
Other:	Thomas Reese	Chief, Functional Neuroanatomy	LNNS, NINCDS

COOPERATING UNITS (if any)

NINCDS, NIH

LAB/BRANCH

Office of the Scientific Director

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: .3	PROFESSIONAL: .3	OTHER: none

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sponge aggregation factor (AF), the large glycoprotein complex involved in species specific aggregation is the first aggregation factor to be visualized on the surface of aggregating cells. It apparently functions by a single macromolecule bridging between two cells with some arms extending to one cell and some to another. Many such linkages ligate adhering cells. It is a model of specificity of cellular interactions.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Aggregation factor (AF) is a 18 x 10^6 Dalton glycoprotein which species-specifically causes marine sponge (Microciona prolifera) cells aggregate to cause physiological adhesion. This aggregation factor is the only purified huge glycoprotein for which a biological assay exists and the sponge aggregation system which is most dramatically dependent upon one factor for cellular cohesion.

It is a fibrous macromolecule with a circular filament for a backbone and about sixteen filaments radiating outwards in a sunburst configuration. These experiments are to discover where on the macromolecule are the sites which bind to cells and where are the sites which bind to other AF macromolecules. They are also designed to show where on the surface of the sponge cell the factor binds and if there are specialized zones of adhesion.

Methods Employed: Microciona prolifera, freshly collected by the Supply Department of the Marine Biological Laboratory, Woods Hole, MA, were dissociated by soaking in calcium and magnesium-free sea water, a procedure which liberates aggregation factor (AF) into the supernatant. AF was further purified by differential centrifugation and chromatography.

Cells returned to complete sea water in a gyratory shaker aggregate upon the addition of AF.

Dissociated cells, aggregating cells, and purified factor were rapidly frozen, freeze fractured and replicated or freeze substituted, stained en bloc with $HfCl_4$, embedded and sectioned for transmission electron microscopy.

Major Findings: Aggregation factor was visualized by transmission electron microscopy on the surface of aggregating sponge cells.

Dissociation of cells was achieved by divalent cation depletion, which extracts AF. The surface of these cells appear remarkably smooth. However, surfaces of aggregating cells to which an excess of purified AF (to be certain of having enough to visualize) and 10^{-2} M $CaCl_2$ were returned have a filamentous network over much of their surface.

In sections and replicas at fortunate angles, the distinctive filamentous configuration of AF, a circular backbone with about 16 arms radiating outward, can be seen. The circular backbone is sandwiched between adjacent cells with some arms extending to one cell and some extending to the other. Arms can also contact other AF macromolecules.

Examination of aggregation of cells with an excess of AF shows that:

1. AF macromolecules bind to the cell surface during aggregation.

2. At a given site a single AF sunburst complex may suffice to link adjacent cells but many such linkages exist between cells, and,

3. Sites in the chains of AF bind it to cells.

Rigor demands examination of stereo pairs of replicas of cells aggregating in a minimal concentration of AF to sustain the observations more easily made with excess AF. Careful observations of stereo pairs support the understanding that each factor macromolecule binds some chains to one cell and other chains to another cell.

Significance to Biomedical Research and Program of the Institute: Cellular surface specificity, which allows cells to recognize their appropriate place in an assemblage of cells, is essential to proper morphogenesis and histogenesis, for example, the awesome specificity of neural synapses. Such surface specificity of cells is no less important to adult functioning of cells and tissues and their misfunctions in cancer and injuries.

Proposed Course of the Project: This project will be terminated after publication of results. The manuscript is in preparation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00152-02-OSD

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Ectopic Osteogenesis Ultrastructure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	OSD, NIA
Other:	James T. Irving	Expert	LCMB, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Scientific Director

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Osteogenesis induced by subcutaneous implantation of bone extracts in rodents follows the ultrastructural pattern expected for normal bone formation and produces fully mineralized bone.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The objective is to assess the normality of osteogenesis during ectopic bone formation, utilizing conventional microscopical methods.

Methods Employed: Ectopic bone was induced by subcutaneous implants of bone or tooth powders in Wistar NIA rats. Induced bone was excised for microscopy at various time intervals after implantation until stages where the extracellular matrix was too mineralized to cut. Standard fixation by immersion into glutaraldehyde-formaldehyde in cacodylate buffer and post fixation in osmium tetroxide and embedding for sections for electron microscopy were employed.

Major Findings: As far as we have been able to ascertain, ectopic bone formation in the rodent proceeds indistinguishably from normal bone formation, once the osteoblasts have differentiated sufficiently to be recognizable as osteoblasts. The source of the cells which become osteoblasts has yet to be determined.

Significance to Biomedical Research and Program of the Institute: Results thus far suggest that the mechanisms involved in the formation of ectopic bone, induced by powdered bone or other promoters, are no different from those in normal osteogenesis. This suggests the possibility of inducing and controlling new bone formation in situations where bone mass is deficient, e.g., osteoporosis.

Proposed Course of the Project: No work was done on this project during this period. No further work is planned.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00153-02-OSD

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Brush Border Ultrastructural Alterations with Ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	OSD, NIA
Others:	Uzi Reiss	Visiting Fellow	LMA, GRC, NIA
	Bertram Sacktor	Chief, LMA	LMA, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Office of the Scientific Director, NIA

SECTION

INSTITUTE AND LOCATION
Experimental Morphology Section

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.25	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Regeneration of the brush border of the proximal kidney tubule after isolateral ischemia has been monitored by transmission electron microscopy. The brush border takes longer to reform than does restoration of full activity of three enzymes (alkaline phosphatase, maltase, and γ-glutamyltranspeptidase) used as biochemical markers of brush border integrity.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The objective is to give a structural basis to the biochemical findings of Dr. U. Reiss and Dr. B. Sacktor in the Laboratory of Molecular Aging. Biochemical extracts of isolated brush border of the proximal tubule show that activity of alkaline phosphatase, maltase, and γ-glutamyltranspeptidase decline after isolateral kidney ischemia. Morphologic examination was performed to determine possible explanations for these declines in activities and to discern relative roles of de novo synthesis and reorganization of the brush border in the reformation of brush border, and how this reformation might vary with age.

Methods Employed: Kidneys were removed from rats after temporary ischemia induced by unilateral renal artery occlusion and blood reflow and were put into cold saline. Portions of the cortex and outer medulla of the mid-kidney were excised and fixed by standard immersion fixation in glutaraldehyde-formaldehyde in cacodylate buffer, postfixation in osmium tetroxide, and were then processed for transmission electron microscopy.

Major Findings: Our findings paralleled those of Venkatachalam, et al. (Kidney Intern. 14, 31-49, 1978) except for the delayed recovery after our longer (1 hour as opposed to their 25 minutes) ischemia. The tubules collapse and show degenerative changes. The microvilli swell, coalesce, and lose parallel order. The microvilli were largely internalized, although some were sloughed off into the lumen along with blebs of cytoplasmic material.

Other cytoplasmic changes included mitochondrial damage, cytoplasmic swelling and formation of large vesicles. In addition to the luminal vesicles and infoldings characteristic of S_1 and S_2 regions of the proximal tubule, relatively translucent vesicles, usually considered lysosomes, are present in cells of all regions of the proximal tubule. After 3 hours reflow following 1 hour ischemia, these lysosomal vesicles are dense and contain membrane elements, at least in those cells which appear to be recovering in S_1 and S_2. The basement membrane apparently remained intact during all the experiments.

The lumina are completely impacted with sloughed material and dying cells until about 3 hours after reflow. Then the S_1 and S_2 portions are partially cleared but the S_3 portion, so disorganized that it is difficult to recognize, remains largely impacted.

The primary question of the source of the new brush border has not been answered. Most, by far, of the brush border is coiled and internalized. However, there is no obvious realignment of this membrane and extrusion. The microvilli of regenerating cells have exaggerated dense tips beneath the cell membrane where the actin filaments terminate. These exaggerated densities have not been found on the control, nonischemic kidneys, and may be characteristic of de novo microvillar formation.

Some cells in S_1 and S_2 have an essentially normal brush border after 30 minutes reflow, but others lack the border after 3 hours. Biochemical markers, alkaline phosphatase, maltase, and γ-glutamyltranspeptidase, reached full activity by 30 minutes. Thus, complete structural integrity is not necessary for full activity of three enzymes characteristic of the brush border.

The major difference between mature adult and aging rats is that morphologic recovery of the brush border is slower and is encountered less commonly in aging rats.

Significance to Biomedical Research and Program of the Institute: If the reformation of the brush border which occurs in the convoluted proximal tubule is really de novo synthesis, then it offers a system to study how profound the differences in brush border of young and old rats are.

Proposed Course: The morphological data will be further analyzed and compared to biochemical data and the results prepared for publication.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00154-02-OSD

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Programmed Cell Death

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	OSD, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Scientific Director, NIA

SECTION
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INSTITUTE AND LOCATION
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TOTAL MANYEARS: .2	PROFESSIONAL: .1	OTHER: .1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Programmed cell death in gonadal cells of the nematode Panagrellus redivivus occurs in normal development and is genetically controlled. Regulation of programmed cell death may be a model for regulation of aging and death in organisms. We are studying the morphologic sequence of events involved in cellular death.

PHS-6040
(Rev. 2-81)

Project Description:

Objective: Study of programmed cell death, often an essential part of normal embryonic development, has two goals: (1) to understand the regulation of normal cell death, which benefits the organism, as contrasted with cell death occurring in disease and by accident, which does not benefit the organism, and (2) to develop a possible model for studying the nature of degenerative changes which lead to the death of an organism.

Methods and Questions: While embryonic programmed cell death occurs widely in invertebrates and vertebrates, including humans, only in the nematodes has cell death been described for a particular cell identifiable by its position in the organism (Sulston and Horvitz, Dev. Biol., 56, 110-156, 1977; Sternberg and Horvitz, Dev. Biol., in press). Cell death occurs in the cell designated as Z4pp in lineage maps in the female *Panagrellus redivivus* gonad, whereas in the male or in the related genus *Caenorhabditis*, Z4pp becomes the distal tip cell and leads development of posterior structure. Elsewhere, only populations of cells can be described, some of which die and some of which do not die.

Development of living *Panagrellus* will be observed in Normarski optics and staged. Appropriate developmental stages before cell death will be selected and processed for electron mocroscopy to determine the first evidence of impending death. Is it signaled by a lesion in a particular organelle?

If the death is programmed, the mechanism of the program may be indicated by the sequence of predeath changes. These changes may differ from those of a cell "murdered" by injury. The question of mechanism has been examined in other systems, but because of the confusion inherent in populational events, and perhaps because of multiple causes and pathways of cell death, there is no agreement.

If it is possible to microinject into or onto the cell fated to die, it is then possible to attempt to prevent death with growth factors or factors from the homologous cell in the male which does not die.

Characteristics of the cell surface are of significance because the surface interacts with other cells and hormones. The surface will be studied by freeze fracture and deep etching. If we are technically able, lectins and receptors will be labelled with ferritin, microinjected, and observed in replicas and in the scanning electron microscope. Quantitative and qualitative changes will be noted and compared to comparable cell in the male which does not die.

Currently, *Panagrellus* is being cultured, basic anatomy and staging learned, and microinjection methods explored.

Significance to Biomedical Research and Program of the Institute: The basis of normal cell death is being explored as an example of time-dependent regulation and as a model of the nature of aging.

Proposed Course of the Project: Exploration of death events and microinjection methods will be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00155-01-OSD

PERIOD COVERED
October 1, 1980-September 30, 1981

TITLE OF PROJECT (80 characters or less)

Morphologic Analysis of Cardiac Muscle Used for Speckle Analysis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	EMS, OSD, NIA
Others:	Edward G. Lakatta	Chief, Cardiovascular Section	CPB, GRC, NIA
	Arthur A. Kort	Clinical Associate	CVS, CPB, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Office of the Scientific Director

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Laser light passing through cardiac muscle fibers exhibits scattered light intensity fluctuations (slif) which appear as dancing speckles of light upon a screen. Physiologic experiments correlating slif to calcium concentrations were performed after detergent treatment. Fibers from these experiments were examined by electron microscopy for membrane integrity and other features which may be correlated to changes in slif.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To determine fine structural changes in cardiac muscle which are caused by certain detergent treatments and which change scattered light intensity fluctuations (slif) in order to gain a better understanding of the functional significance of the slif phenomenon.

Methods Employed: Cardiac fibers, ligated to be at a constant tension during detergent extraction and laser light scattering, were fixed by sequential immersion with glutaraldehyde and osmium tetroxide and embedded, sectioned, and examined by electron microscopy by standard techniques. Controls were ligated fibers without detergent extraction and fibers from a rat perfused with fixative.

Major Findings: Detergent "skinning" of muscle fibers stops slif. In the most interesting situation, saponin dissolves most of the sarcolemma but not the sarcoplasmic reticulum, which is swollen. This dissolution of the sarcolemma permits diffusion of substances into the fibers to alter the slif. Other observations include shorter sarcomere length in the ligated samples than in perfused muscle and greatly accentuated appearance of myofilament cross bridges in saponin treated fibers.

Significance to Biomedical Research and Program of the Institute: Slif is a new means of examining muscle and has been correlated by Lakatta and Kort to calcium concentrations in detergent "skinning" experiments. Appropriate changes in calcium concentrations in cardiac muscle are essential for heart muscle contraction and relaxation. As many as 25% of heart attacks may be caused by improper calcium currents. What slif can tell about these phenomena is yet to be learned.

Proposed Course of the Project: Additional morphological work will be done when it can aid interpretation of physiological experiments.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00156-01-OSD

PERIOD COVERED
October 1, 1980-September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization of a Receptor to Palladin, the Aggregation Factor of Polyspondylium pallidum

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	EMS, OSD, NIA
Others:	Dr. Steven Rosen	Associate Professor	Dept. of Anatomy University of California at San Francisco
	Ms. Debra Drake	Research Associate	Dept. of Microbiology University of California at San Francisco

COOPERATING UNITS (if any)

Departments of Anatomy and Microbiology, University of California at San Francisco

LAB/BRANCH
Office of the Scientific Director

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pallidin, a cell surface lectin implicated in mediating cell-cell adhesion in the cellular slime mold Polyspondylium pallidum, is inhibited by an endogenous factor which binds to it. The inhibiting factor, apparently a receptor, appears as a ring structure in the electron microscope.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: We are dissecting the components of the cell surface to discover the molecular basis of physiological cohesion.

Methods Employed: S. Rosen and D. Drake isolated a receptor identified as a potent hemagglutination inhibitor of pallidin, by affinity precipitation with pallidin and gel filtration. I prepared this large (at least 10^6 Daltons) glycoprotein for electron microscopy by (1) low angle deposition of a 10 Å platinum shadow, and (2) uranyl acetate staining.

Major Findings: The putative receptor is a fibrous ringed structure about 400 Å in diameter. The fiber is about 110 Å in diameter. After freezing and thawing, this ring seems to fray, so revealing an apparent structure, possibly arising as a consequence of lateral aggregation of chains of rings.

Significance to Biomedical Research and Program of the Institute: Surface specificity of cells of cohesion is critical to establishment and maintenance of intercellular functions. A knowledge of the components of cohesion is fundamental to manipulating cohesion in cancer and tissue injuries.

Proposed Course of the Project: Several permutations in preparation for electron microscopy will be performed to define the substructure of the macromolecular complex. Binding sites for pallidin will be discerned by examination of complexes of pallidin-receptor. Preliminary work will be reported at the meeting of the American Society for Cell Biology in November.

Publications: None.

Laboratory of Behavioral Sciences

One of the most fundamental questions in the biological sciences concerns the nature of the processes which mediate plasticity and those which maintain stability. These processes operate at the level of the single cell, in organ systems, in entire organisms and even in social units. Clearly, both stability and plasticity are essential to survival, yet these processes must remain in balance. Three projects which looked at these issues found considerable evidence for age related, stabilizing effects. One study has shown through the use of personality assessment procedures that major personality characteristics such as neuroticism, extraversion or openness to experience remain stable throughout life, and that this stability also can be seen in spouse ratings as well as in self-reports of personality. The second project looked at age differences in autonomic response patterns to a battery of stimuli and found that older subjects showed greater response consistency--i.e., less plasticity--than did younger subjects. This continuum was seen over the age range 20 to 80 years. A third project which is still at an early stage of development is designed to create a behavioral test battery for laboratory rodents. One set of tests designed to evaluate motor behavior already has shown not only age effects but also genetic effects: one group of mice characterized as short-lived showed clear cut, age-related declines in performance of simple motor tasks requiring upper body strength and no age differences in locomotor performance, whereas a long-lived group of animals showed the opposite aging pattern. Subsequent measures which will be included in the test battery will enable us to evaluate sensory processes, learning, motivation and even problem solving abilities. These studies will be supplemented with biochemical and neurophysiological analysis designed to identify some of the mechanisms underlying these effects.

Despite serious limitations on program imposed by personnel constraints, LBS continues to maintain one of the foremost programs in the field of Behavioral Medicine in the world. Several major projects were completed this year. These, and some which are continuing, illustrate our leadership. One project assessed the oft-repeated assertion that cigarette smoking is a behavioral device for coping with stress. Comparisons of the psychological well-being of smokers and non-smokers showed that smoking neither reduces negative affect nor enhances positive affect. Rather, smoking primarily reduces the distress of nicotine deprivation. The so-called psychological benefits of smoking appear to be rationalizations. In a major program designed to evaluate behavioral interventions in the control of elevated blood pressure, we have found that: 1) self-monitoring of blood pressure is feasible and clinically beneficial, i.e., associated with significant falls in blood pressure; 2) the behavioral procedures of systolic blood pressure biofeedback and a combination of progressive and meditative relaxation will reduce pressure beyond that due to self-monitoring; 3) patients who first use the feedback procedure and then the relaxation procedure achieve the greatest benefit (overall reductions of about 10% systolic and diastolic pressures--about 142/92 to 130/82 mm Hg--in a period of seven months). This laboratory also operates major programs in the study of behavioral treatments of fecal and urinary incontinence. One project which is ongoing has found that in a consecutive series of nine spina bifida children suffering from lifelong fecal incontinence, it has been possible to train seven of the children either to become totally continent or to have fewer than one incontinent episode/month. In a related, on-going project we have found that we could successfully retrain four of five urinary incontinent, and four of six fecally incontinent elderly adults (age range 65 to 88 yrs), and that once retrained these patients then were able either to be totally continent or to be able to reduce their incontinent episodes by 70% or more.

There are many ways to measure complex, intellectual skills. Several studies carried out over the past year illustrate this fact. In one study in progress, cognitive complexity is manipulated experimentally. The perceptual and motor components are held constant in a series of five response-time tasks, and complexity is manipulated by varying the cognitive demands of the tasks. The data for men and also for women indicate that age differences increase with increasing complexity. The smallest increases in response time with age are emerging for the simplest task and the largest increases with age are found for the two most complex tasks. These data suggest slowing in central processing with age. An earlier study in this laboratory showed that the number of task-unrelated, thought-intrusions (daydreams) declines with age as measured by a retrospective questionnaire. Research now in progress is showing that this effect also can be seen in an experimental test of vigilance: daydreaming declines with age.

Still another way of looking at complex behavior is to ask how ability to cope with various life stresses is determined. Two studies completed this year were designed to assess the roles of the type of stress, age, sex and personality on coping ability. Some of the major findings were: 1) most individuals facing challenging situations use the coping mechanisms of rational action, perseverance, restraint or humor; 2) individuals facing threats to health, safety or security may use rational action, but they also frequently rely on faith or wishful thinking; 3) there are clear age differences in the nature of life stresses with older subjects experiencing more threats to health, safety or security; 4) when young and old subjects are compared in terms of their coping responses to threats, the younger subjects are seen to be more likely to use "immature" coping mechanisms such as hostility, escapist fantasy or wishful thinking, whereas older subjects are more likely to use "mature" strategies such as rational action; 5) personality traits tend to influence coping processes, e.g., neurotic subjects are more likely to respond to stresses with hostile reactions, extroverts are more likely to persevere, persons who are open to experience are likely to rely on humor and closed persons will tend to use faith to cope. It is possible to study complex, intellectual processes in a basic science, animal laboratory. Previous research in LBS showed consistent declines in long-term memory in aged rodents. Research this year has shown age-related declines in short-term, spatial memory in the rat. Furthermore, neurochemical analyses have shown that these declines are correlated with the activities of the enzymes choline acetyltransferase in the hippocampus (which is an essential structure in spatial memory) and glutamic acid decarboxylase in the cerebral cortex.

This laboratory has made several important contributions to the understanding of the age-related changes in behavior associated with changes in central neural transmitters. Most of our research has concentrated on the nigrostriatal, dopaminergic system which is strongly involved in the central neural processes which underly Parkinsonism in man. Previous research in our laboratory had shown consistent age-related deficits in the number of dopaminergic receptors in the striatum. However, until this year it was not possible to show conclusively that this deficit explained the age differences in the striatum-mediated motor behavior we observed consistently. In a series of experiments this year it was shown that: 1) L-DOPA pretreatment did not enhance amphetamine-induced rotational behavior (a test of dopamine-mediated activity) even though tyrosine hydroxylase and dopa-decarboxylase (enzymatic precursors of dopamine) were unchanged, and striatal dopamine uptake and release mechanisms also were unchanged indicating that L-DOPA was not converted to dopamine in the old animal; 2) even though senescent rats have fewer receptors, lesions produced by 6-hydroxydopamine did not differentially increase the number of receptors in young and old animals; 3) when dopamine or amphetamine was injected directly into the striatum the effect on rotational behavior was less in older animals than in younger animals showing that the primary age-deficit probably was in receptor availability, not in transmitter production. Future research in this program will examine some of the interactions between the cholinergic and dopaminergic systems, studies on the serotonergic neurotransmitter system will be implemented, and studies on age-differences in the endorphin/encephalin system will begin.

Previous research in LBS has shown that rats which have their diets restricted by alternate days of feeding and fasting live about 40% longer than do ad-libitum fed control animals. Studies of dopamine receptor concentrations in these animals now have shown that periodically fasted, 24 month old animals have about 50% more receptors than do age-matched controls. The receptor density in these fasted animals is almost as great as is the density in 3-6 month old rats.

There are several model systems for studying the relationships among physiological response systems, neural regulatory systems and behavioral control systems. One such model system is the cardiovascular adjustments to exercise, a second is the so-called central command system by which complex peripheral response patterns are emitted through the mediation of central neural "plans," and a third is the interaction between exercise and nutrition in the determination of longevity. Several, interesting studies have been carried out on each of these problems within the past year. Previous research in LBS showed that normal subjects can be trained to attenuate the tachycardia of exercise while exercising at mild levels on a bicycle ergometer. Research this year has shown that this is possible even when the subjects (in this case conditioned athletes) perform at exercise loads designed to induce heart rates of 65% of maximal heart rate. These findings show that voluntary control of cardiovascular function is possible even under conditions where heart rate is being mediated predominantly by way of the sympathetic nervous system. Other studies carried out in these subjects showed that the learned effects were associated with cardiovascular and/or pulmonary adjustments but not with peripheral motor adapatations since the decrease in heart rate was associated with a decrease in oxygen consumption but no change was seen in blood lactate levels. In 1970 we developed a nonhuman primate model of learned cardiac control. Two experiments completed this year have shown conclusively that heart rate control in this preparation is mediated through highly sophisticated, central neural modulation of central neural control of patterns of cardiovascular responses. In one experiment we showed that when monkeys were modulating their heart rates, they are inhibiting or attenuating baroreceptor-mediated responses which might otherwise interfere with the cardiac control. In a second experiment we showed that monkeys which were trained to control heart rate could override the effects of direct brain stimulation while controlling their hearts: e.g., direct electrical stimulation of the posterior hypothalamus produces a tachycardia and a pressor response; when a monkey is trained to slow its heart it will block the tachycardia but not the pressor response. This effect is not present when the anterior hypothalamus is stimulated to yield a tachycardia and a pressor response. These experiments clearly show that central neural command of cardiovascular responses are plastic and in some cases, at least, can be brought under voluntary control. Both exercise and periodic deprivation have been associated with significant increases in longevity in the rat. Research completed within this year has shown clearly that only periodic fasting increases longevity. Exercise increases longevity secondary to its effect on weight reduction. Animals which are periodically fasted and exercised do not live longer than rats which are fasted but not exercised. Other studies in this program have shown that the effect of periodic fasting is complex and is not due merely to prolongation of the growth phase of life.

Although the scientific productivity of LBS has been consistently high, this laboratory will have serious problems in the future if it does not receive additional personnel support. During this year Dr. Charles Goodrick died. Dr. Goodrick was the foremost investigator in the world in animal behavior and aging. His loss is a personal sorrow for the staff of LBS, but it also creates a very serious professional deficit in our programs. If it is not possible to replace him, the GRC may lose its place at the forefront of research on animal behavior and aging. In addition to the threat to our role as an international leader in research on animal behavior and aging, our position in Behavioral Medicine also will be in jeopardy if we cannot recruit additional members to our staff. For example, our finding that behavioral intervention can significantly lower blood pressure in mild to moderately ill patients with high blood pressure should be further tested in a more severely ill cohort which also will be an older cohort. If our findings can be extended to these patients, they will have an important impact on the care of these older, sicker people. However, unless personnel resources become available, this extension of our program can not be implemented.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00061-19 LBS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Behavioral Genetics and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
OTHERS:	Donald Ingram	Staff Fellow	LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning and Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to determine behavioral differences throughout the lifespan for populations of mice which differ in genetic structure, and which also differ in longevity. The present topics of interest concern the relations of growth rate, maximal body weight and longevity for mice fed low levels of dietary protein and for groups fed adequate dietary protein, in addition to studies of aging and voluntary wheel exercise, and ethanol preference.

Research previously included in this project and still continuing is reported now in Project Z01 AG-00063-14 LBS.

PHS-6040
(Rev. 2-81)

Project Description:

Combined into Project Z01 AG-00063-14 LBS.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG 00062-08 LBS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Daydreaming and Aging: Normative and Experimental

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard M. Giambra	Research Psychologist	LBS GRC NIA
Other:	Bernadette Cider		Towson State Univ.
	Eric Klinger	Prof. of Psychology	University of Minnesota
	Reginald Quilter	Electronics Tech.	LBS GRC NIA
	Jan Sinnott	Associate Prof. of Psychology NRSA post-doctoral fellowship	LBS GRC NIA

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning & Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.8	.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to determine the parameters of spontaneous-thought-intrusions (daydreaming) and related mental activity such as insight, intuition, mindwandering, and curiosity. This is accomplished through the use of retrospective questionnaires and experimental manipulation. Topics of present interest are: (a) the lifespan changes in daydreaming in a longitudinal sample, (b) the relation of sexual daydreaming and sexual activity to the menopause, (c) the relation of daydreaming incidence to tasks of varying vigilance requirements, and (d) the dimensions of ongoing thoughts on a lifespan sample.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The goals are: (1) to determine the incidence and content of daydreaming in specific subpopulations (e.g., young, middle aged, elderly) from various socio-economic classes, various races, etc; (2) to attempt to relate these differences in daydreaming to any underlying mechanisms such as physiological state, education, cultural values and beliefs, differential daily experiences, and (3) to investigate experimentally variables which normative studies have indicated may be potent determiners of daydreaming.

Methods Employed: The normative aspects of daydreaming are determined through the use of a structured self-report. Each participant completes a 21 item biographical questionnaire and a 344 item Imaginal Processes Inventory (IPI) which has both specific and general items concerning daydreams, nightdreams, fantasies, etc. There are 33 factors in the IPI. Each item has five choices which are points on a continuum implying frequency or quantity. The choices were assigned values of 0, 1, 2, 3, or 4.

In a vigilance task, the subject must detect changes in visually or auditorially presented material. For example, the subject may see a stimulus designated "A" flash on a projection screen at a specific rate; sometimes, however, another stimulus designated "B" will occur instead of A. The subject's tasks are to detect and report when B appears and to report, by means of a button press, the occurrence of all task-unrelated-thought intrusions. The proportion of B's can be controlled as well as the number of stimuli presented per minute and the interval during which stimuli remain in view. These factors determine the difficulty and tediousness of the vigilance task and are expected to be related to the incidence of daydreaming.

A second example of a vigilance task used is the Mackworth Clock (MC). The MC is of one hour duration. The participant is told to monitor a clock-like pointer which moves in discrete steps once every second. After relatively long, varying periods of time, the clock's pointer describes a continous double step in the one second period. The participant is asked to press a push-button, as quickly as possible, as soon as the "double jump" is perceived.

In the thought sampling task each person carries an alarm device (a beeper) which emits a soft tone at random intervals with a mean of 40 minutes. Each time the beeper sounds the person stops what he or she is doing, records the last thought which occurred when the beeper sounded and rates that thought on 24 scales of a Thought Sampling Questionnaire. The beeper is carried with the person during day-to-day normal activities. Each person provides about 150 thought samples.

I. Vigilance and daydreaming incidence.
The vigilance task described earlier is being used to measure directly the incidence of daydreaming and mindwandering for people across the life-span. A life-span sample is being used since the self-reports of subjects, based on the Imaginal Processes Inventory, suggest that with increasing age there is an increased ability to concentrate on any task without the intrusion of daydreams. A series of studies investigating the effects of various parameters of the vigilance task and of the subjects themselves on the frequency of daydreaming and mindwandering has begun. The first study varies the length and tedium of the vigilance task. In this study so far, about 56 longitudinal participants have participated. They ranged in age from 28 to 83 years old. Analyses based upon

this small sample indicated that daydreaming and mindwandering do seem to drop after age 60. Data collection continues.

The Mackworth Clock vigilance task described earlier will also be used in a longitudinal study of subjects who participated in the identical study in 1962-1964. Measures of reaction time and galvanic skin response will also be taken. These same subjects will receive the first vigilance task and thus will allow correlations to be made among the present tasks as well as with tasks performed in 1962-1964. A total of 32 men have participated who participated in 1962-1964. In addition 40 men aged 30-89 have participated for the first time.

II. Dimensions of the stream of thought across the life span.
This study has experienced great difficulty in obtaining volunteers for participation. Presently only one 36 year old female and one 37 year old man have participated. Recruitment of subjects has been suspended.

III. A longitudinal study of daydreaming and related mental activity.
Starting in 1979, men in the Baltimore Longitudinal Study of Aging again began completing the IPI. Presently 203 men who took the IPI 6-8 years ago have been retested and also 161 men have been tested for the first time. This data collection will continue until the entire BLSA population of men has been completely tested or retested. In 1980 the women in the BLSA began to take the IPI. Presently 107 women have taken the IPI. A subsample of these women participated in a separate study in 1975 and therefore represent a longitudinal repeat subgroup.

IV. Sexuality of 40-60 year old women: Relation to health and menopause.
The objective of this analysis is to determine patterns of sexual behavior in middle-aged women of varied health and menopausal status. Another objective is to identify the major determinants of frequency of sexual relations, frequency of masturbation, and interest in sex in this population. This analysis tests the hypothesis that health and availability of a partner more than age or menopausal status affect a woman's sexual behavior. It is also expected that women in this age range will present a variety of patterns of sexual behavior at any age, and that "menopausal" symptoms will have little relation to menopausal state or sexuality.

More than 477 women from the Baltimore and St. Louis Metropolitan areas participated in a study on sexual daydreaming. They also responded to a structured paper-and-pencil questionnaire which measured sexual behavior, attitudes, health, and menopausal symptomatology, menopause status, and demographic variables including age. Menopausal symptomatology was measured by questions drawn from widely-used scales.

Results demonstrated that healthy women in their 50's with no gynecological problems who had willing partners did not differ from women in their 40's in sexual behavior. "Menopausal" symptoms were not related to menopausal status, but were found in the greatest numbers in women with poorer health and painful menstruation. In the entire sample, healthy or not, frequency of intercourse was most strongly related to health, presence or absence of hot flashes, partner's interest, log of time since last menstrual period, and perceived importance of being a sex partner ($R^2 = .21$). Age did not account for significantly more variance than the variables listed above. Women's interest in sex (in comparison to partner's interest) was related to perceived importance of being a sex partner and perceived importance of being a homemaker.

V. Daydreaming, health, and menopausal status in 40-60 year old women. Giambra (See 1976-77 annual report) in a cross-sectional study of daydreaming from the late teens to the early nineties observed two midlife influences on daydreaming. In the first, there occurred for both men and women in the 45 to 49 year old age span a very large increment in daydreaming frequency and absorption in daydreaming. For daydreaming frequency this increment was directly counter to the trend of a steady drop for both younger and older segments of the age function. Absorption in daydreaming was much greater than that shown by those cohorts one-half decade older and younger. In the second, the scale which indicated the extent to which daydreams were of a sexual nature showed a very large drop at age 50 for women. That is, from age 35 to age 49 years sexual daydreaming scale mean values were unchanged for each half-decade while at age 50 to 54 the sexual daydreaming scale mean values dropped by 32%.

For women the period from 40 to 60 years of age contains a most important biological event, the transition to menopause. Menopause, the cessation of menses, occurs for most women in the age range from 45 to 54 years. This transition, as well as the final result, is accompanied by a clear change in the hormonal balance of the women. Thus it may be fruitful to explore the extent to which the physiological changes which occur in 40 to 60 year old women may be related to the observed changes in daydreaming.

The menopausal transition does not occur in a vacuum. Psychological, sociological, social, familial, and economic changes may occur in women from 40 to 60 years of age. Changes in marital status brought on by divorce or widowhood, the disappearance of children from the home, the need to care for aging parents, the desire to expand one's perspective by obtaining a job or an education, are all events which may accompany the menopausal transition. Daydreaming, which may be defined as a spontaneous intrusion into awareness of a thought unrelated to the task at hand, may reflect these changes. Furthermore, these changes often engender strong emotions and may represent important unresolved issues in the lives of these women. In Psychologist Eric Klinger's terms they represent the "current concerns" of 40 to 60 year old women. The current concerns, being both highly charged emotionally or in the process of resolution, are viewed as imbued with an autonomous dynamism which would have a high priority within the normal problem solving function of the mind. Thus one would hypothesize that an increase in daydreaming frequency would be the result of these current concerns. The purpose of this study is to examine in a much larger sample the daydreaming characteristics of women 40 to 60 years of age in an attempt to: (a) replicate the earlier finding and (b) determine the extent to which the menopausal transition and other midlife non-physiological changes may be related to the observed daydreaming characteristics.

A total of 477 women were sampled in 1977-78: 67 were 40-44 years old, 114 were 45-49 years of age, 159 were 50-54 years of age, and 137 were 55-60 years old. All were volunteers who were paid $5.00. Most of the women were members of volunteer service organizations in the Baltimore, Md. and St. Louis, Mo. metropolitan areas who donated the $5 payment to their service organization. The women did not know beforehand that they were to be questioned about daydreaming. The sample may be described as follows: (a) Race, White 97.6%, Black 1.2%, other 1.0%; (b) Marital Status, married 87.1%, divorced 4.2%, separated 1.2%, widowed 5.8%, never married 1.4%; (c) Joint or Family Annual Income, $10,000 or less 7.7%, $10,001 to $20,000 30.6%, $20,001 to $30,000 29.4%, $30,001 or more 32.0%; (d) Residency, farm 1%, rural area 12.1%, town/city of 50,000 or less 8.7%, city of 50,001 to one million 17.9%, city of more than one million 42.5%, other 17.5%; (e) Religion, Protestant 19.6%, Jewish 58.6%, Catholic 18.5%, other 3.0%; (f) Education, 9th grade or less 4.2%, grades 10 to 12 54.1%, college 1-2 years 22.7%, college 3-4 years 10.8%, graduate study 7.8%.

For purposes of analysis the "total" sample was reduced into two successively more selective subsamples. The first or "married" subsample was restricted to all those in the total sample who were married and living with their spouses, who were White, and who described themselves as being in excellent, good, or fair health. The second or "no-problems" subsample was restricted to all those in the married subsample who did not (a) visit a physician or specialist less than six months ago for a gynecological, endocrine, or psychological problem, or (b) have a complete or partial ovarectomy or hysterectomy, or (c) takes drugs for gynecological, endocrine, or nervous disorders, or for "change of life." The "married" subsample provided a relatively homogeneous group where demographic variables might have a minimal effect. The "no-problems" subsample provided a relatively healthy group with regard to gynecological, psychological, and endocrinal disorders. This was done with the intent of determining the extent to which these health problems moderated any daydreaming relationships found with 40 to 60 year old women.

A short form of the Imaginal Processes Inventory was used. The shortened IPI was designated the Mental Processes Short Inventory (MPSI). The MPSI consists of 84 items which form 13 scales. Each scale, except one, consists of the 6 items of the original IPI scale which, for a life-span sample of women, correlated the highest with the original scale score. The exception was that all 12 items of the IPI Sexual Daydreaming scale were used.

An Informational Questionnaire was also used. The information requested was concerned with five areas of interest: demographic variables (see sample description above), health and gynecological variables, sexual activities, importance of various activities, and effects of menopause. Informational questions were included with the expectation that they might be important moderator variables with regard to daydreaming in general or sexual daydreaming in particular in the 40 to 60 year old age groups. The Informational Questionnaire took from 1/2 to 1 hour to complete.

Menopause as an Effect

Menopause may be viewed as a purely physiological event or in terms of the extent to which one exhibits the "common symptoms" of menopause. Physiologically, menopause occurs with the cessation of menses. It is common to define post-menopause as having occurred when menstruation has ceased for 13 or more months. Likewise, women may be classed as pre-menopausal if they have menstruated within the previous four months. Women who have not menstruated in the previous five to 12 months were categorized as menopausal.

Menopause is often accompanied by an array of somatic and psychological symptoms of varying severity. A measure of the effect of menopause on a woman can be quantified by arranging these symptoms into an index or scale. One such commonly used index is the Blatt Menopause Index. A modified form of the Blatt Menopause Index was used in this study. Blatt used 12 symptoms, each rated on a severity dimension. The symptoms which are particularly salient to menopause were more heavily weighted. The menopause index in this study used eleven symptoms each weighted using Blatt's weights and additively combined. No measure of the severity of the symptom was asked; the symptom was checked when it occurred "regularly."

Since age has been shown in this laboratory to be related to daydreaming, age was used as an independent variable and an Age (45-49, 50-54) X Menopause (Pre-, Yes, Post-) ANOVA was performed on each of the 13 scales of the MPSI. The cells in these and subsequent ANOVA's do not contain equal numbers of observations and since this was not indicative of population differences, unequal n, harmonic mean ANOVA's were performed. In these ANOVA's age was restricted to the 45-54 year old groups since there were so few women who were [peri-] menopausal under 45 and over 54 years of age. The following scales were significantly ($p < .05$) affected by the Menopause variable in both the total sample and married subsample: Daydreaming Frequency, Fear of Failure Daydreams, and Achievement-Oriented Daydreams. The Age X Menopause interaction was significant for the Daydreaming Frequency, Absorption in Daydreaming, Problem Solving, and Hallucinatory-Vividness Daydreams scales. The 45-49 year old menopausal women had much larger means than the pre- and post-menopausal women on all scales except Fear of Failure Daydreams. The 50-54 year old menopausal women were like the 45-49 year old women only on Achievement-Oriented Daydreams. On the other scales the 50-54 year old menopausal women were like the pre- or post-menopausal women. The main effect of menopause on Fear of Failure Daydreams indicates that the means increase as groups shift from pre-menopausal to menopausal to post-menopausal. In the no-problems subsample no significant ($p > .05$) menopause effects were noted, but the means mirrored the trends in the total sample.

An additional set of ANOVA's were performed using all four age groups and only the pre- and post-menopausal women. For the total sample the Menopause variable had a significant ($p < .05$) effect on Fear of Failure Daydreams and Problem Solving Daydreams; in both scales post-menopausal women had a greater mean than pre-menopausal women. An Age X Menopause interaction effect was significant, ($p < .05$) for Problem Solving Daydreams, Sexual Daydreams, and Daydreaming Frequency. These interactions took the form of greater means for post-menopausal women in the 40-44 and 55-60 year old groups and greater means for the pre-menopausal women in one or both of the 45-49 and 50-54 year old groups.

When the ANOVA were performed on the "married" and the "no-problems" subsamples only the menopause effect on the Fear of Failure continued to be significant; however, the relative positions of the means of the two menopause variable groups continued.

The Menopausal Index was correlated with the 13 scales of the MPSI to determine the degree of linear relationship between overall symptom presence or complaint and daydreaming. The correlation with the Fear of Failure scale was .35 and all others were below .30. All correlations were substantially reduced within the "no-problems" sample. The modest correlations might have been due to non-linear relationships between the scales and the Index. Hence, women with the following Index values were grouped together: 0, 1-2, 3-4, 5-7, 8-17. Age X Index ANOVA's were then performed on each scale. All scales except Acceptance of Daydreaming and Self-Revelation showed a significant ($p < .05$) Index effect. An examination of the scale means for the Index intervals showed that the 3-4 and 5-7 intervals were nearly the same in magnitude or that the 1-2 interval mean had a magnitude less than the mean for the Index value of zero. These nonlinearities suggested that the effect on the daydreaming scales of each individual symptom be investigated.

The Effect of Regularly Occurring Symptoms

In addition to the eleven symptoms of the Index the Informational Questionnaire asked about eleven additional symptoms, for a total of 22. An Age X Symptom (Present, Absent) ANOVA was performed on each of the 13 scales for each of the

22 symptoms. The results of these ANOVA's are contained in Table 1; also, Table 1 contains a description of each symptom. Table 1 is arranged vertically from the most to the least affected symptom and horizontally from the most to the least affected MPSI scale. The "no-problems" subsample exhibited considerably fewer significant symptom effects than the total sample or the married subsample. An examination of the mean values for the scales indicates that virtually all significant effects were due to higher mean values for the group regularly experiencing the symptom. Furthermore, even in most of the cases where significance was not achieved higher mean values still occurred in the group exhibiting the symptom, although this was much less frequently the case for the "no-problems" subsample. The Acceptance of Daydreaming Scale was usually not significantly affected by the presence of any symptom and, when it was, the presence of the symptom did not consistently act to increase or decrease the mean value; similarly, for the Self-Revelation Scale. It is interesting to note that the presence of the symptom "aches in the back of neck and skull" and/or "headaches" significantly reduced Problem Solving Daydreams in the no-problems subsample.

The Effect of General Health Measures

Four general indicators of health status were obtained. The most general and subjective indicator was obtained from the response to the question "How would you describe your general health?" The four possible responses were: (a) "Excellent--I rarely feel too sick to carry out my regular activities;" (b) "Good--I need to take a few days off two or three times a year;" (c) "Fair--I need to take a few days off four or five times a year;" (d) "Poor--I lose a lot of time due to illness." Three less subjective measures of health were the responses to the following three questions: (a) "How long ago did you last visit a doctor or specialist?" (b) "How often do you visit a doctor or specialist?" (c) "Do you take any medication on a regular basis?"

To determine if age was related to any of these four general health measures each measure had a one-way ANOVA performed on it with age as the dependent measure; this was done on the total sample, the married subsample, and the no-problems subsample. The results of the 12 ANOVA's showed that age was not significantly ($p > .05$) affected by any of these four general health indicators in any of the three groups.

Using the scales of the MPSI as dependent measures each of the four general health indicators had an ANOVA performed on it; the ANOVA's were done on the "total" sample and the "married" and "no-problems" subsamples. In the total sample as the description of health varied from "excellent" to "good" to "fair" to "poor" the mean values of the following scales significantly ($p < .05$) increased: Absorption in Daydreaming, Fear of Failure Daydreams, Hostile Daydreams. Bizarre-Improbable Daydreams and Guilt Daydreams significantly increased as health changed from "excellent" to "fair" then decreased for those with "poor" health. Both Daydreaming Frequency and Achievement-Oriented Daydreams increased as health changed to "fair;" Daydreaming Frequency increased again for "poor" health self-descriptors while Achievement-Oriented Daydreams decreased to the lowest mean value. The same relationships were evident in the married subsample except that the "poor" health category was excluded. In the no-problems subsample only the Achievement-Oriented Daydream Scale was significantly affected by the levels of the general health indicator.

The responses to the question "Do you take any medication on a regular basis?" were categorized into the number of medications taken regularly. The categories used in the one-way ANOVA's were none, one, two, and three to five medications.

Fear of Failure and Hostile Daydream mean values significantly increased as the Number of Medications increased from none to two; mean values for three to five medications were slightly less than for two medications. Acceptance of Daydreams was significantly affected by the number of medications with the means for none to two being the same and three to five being smaller. In the married subsample only Acceptance of Daydreams was significantly affected by Number of Medications; in the no-problems subsample no scale was significantly affected.

The responses to the question "How long ago did you last visit a doctor or specialist?" were placed into five intervals: less than 6 months, 6-12 months, 13-23 months, 24-35 months, 36-59 months. In the total sample only Problem Solving Daydreams was significantly affected with the largest mean in the less than 6 months interval and the smallest mean in the 36-59 month interval and the essentially equal intermediate mean values for the other three intervals. In the married subsample the time-since-last-visit also significantly affected the Fear of Failure Daydreams and the Self Revelation Scales. Fear of Failure Daydreams decreased gradually with increasing interval length while Self Revelation had no systematic relationship to the length of interval. This was also the case in the no-problems subsample where Self Revelation was the only scale where a significant effect occurred. The responses to the question "How often do you visit a doctor or specialist?" were categorized into seven frequency categories. Only Problem Solving Daydreams in the married subsample was significantly affected by this variable. The visit frequency of once a month had the greatest Problem Solving Daydream scale mean; greater or lesser visit frequencies produced smaller means but in no systematic manner.

The Effect of Menstrual Characteristics

Each woman was asked to characterize her menstrual periods as (a) regular or irregular, and (b) not painful, slightly painful, or painful. She also answered the question "Prior to my menstrual periods I am (was): (a) no moodier than usual, (b) somewhat moodier, (c) moodier, (d) very moody." Using Age as the dependent measure and the three menstrual characteristic variables as independent variables, one way ANOVA's were carried out with the result that only Pre-menstrual Moodiness was significant in the total sample; the less moody were somewhat older than the more moody. Using the scales of the MPSI as dependent measures, one way ANOVA's were performed on the Menstrual Regularity and the Menstrual Painfulness variables. Two way ANOVA's using Age and Pre-menstrual Moodiness were also performed using the scales of the MPSI as dependent measures. The Menstrual Regularity variable produced significant effects only in the no-problems subsample with those with irregular periodicity having significantly greater means on the scales Absorption in Daydreams, Hallucinatory-Vividness of Daydreams, and Bizarre-Improbable Daydreams. The Menstrual Painfulness variable produced a significant effect only on the Fear of Failure Daydream scale in the total sample and the married subsample; women with the most painful periods showed the greatest mean.

The Pre-menstrual Moodiness variable significantly affected, in the total and the married subsamples, the Daydream Frequency, Absorption in Daydreaming, Bizarre-Improbable Daydreams, Achievement-Oriented Daydreams, and Fear of Failure Daydream scales. The means in general showed a monotonic increase as moodiness increased. In the total sample the Sexual Daydream scale also was significantly affected by Pre-menstrual Moodiness; the mean values increased to a maximum at the "moodier" category then decreased somewhat in the "very moody" category. The Pre-menstrual Moodiness variable had no significant effects in the no-problems subsample.

The Effect of Sexuality Variables

Each woman answered the following two questions dealing with sexual activity: (a) "On the average, how often do you engage in sexual relations?" (b) "On the average, how often do you presently engage in sexual activity such as masturbation or homosexual relations?" Each question had eight possible responses from "never" to "more than once a day." Using Age as a blocked variable, ANOVA's were performed with each of these variables with the MPSI scales as dependent measures. No significant effects due to Sexual Relations Frequency were observed on any of the scales. The Frequency of Masturbation or Homosexual Relations was found to affect significantly the scales of Daydreaming Frequency, Absorption in Daydreaming, Bizarre-Improbable Daydreams, Sexual Daydreams, Achievement-Oriented Daydreams, and Problem Solving Daydreams. The mean of those who never engage in such behavior is smaller than those that do it with a frequency of once a month or more, i.e., the greater the frequency the greater the mean scale values. The significant effects identified above were observed in the total sample and the married subsample; in the no-problems subsample Bizarre-Improbable Daydreams was not significantly affected. In the married and no-problems subsamples Guilt Daydreams were significantly affected with greater means for greater masturbatory/homosexual relations frequency.

To determine if a change in frequency of sexual relations was related to daydreaming each woman was asked to select one of the options of the following question: "Has this frequency of sexual relations changed within the past few years? (a) No, it's about the same; (b) Yes, the frequency has decreased because i my interest has increased, ii my partner's interest has decreased, iii both our interests have decreased, iv my partner is no longer with me; (c) Yes, the frequency has increased because i my interest has increased, ii my partner's interest has increased, iii both our interests have increased, iv I now have a partner." Age by Sex Frequency Change ANOVA's were calculated using various combinations of the above categories with relatively few significant Sex Frequency Change effects occurring. In most instances these were accompanied by significant age interactions. When means were examined no consistent relationship appeared except that 40 to 49 year old women who have increased their frequency due to "now having a partner" were extraordinarily high in all aspects of daydreaming.

To determine how variation in interest in sexual activity between the woman and her partner was related to daydreaming the following question was asked "Are you more interested in sexual relations than your partner? (a) Neither my partner nor I am interested, (b) My partner is more interested than I, (c) We are both equally interested, (d) I am more interested than my partner." Daydreaming Frequency and Sexual Daydreaming were significantly affected by this sexual interest variable. Daydreaming Frequency means increased as interest moved from "partner" to "both" to the "woman;" however, the "neither interested" category showed the highest mean of all. When Sexual Daydream means were examined, the "I am more interested" category was found to have by far the largest value and the mean values became smaller from "neither" to "both" to "partner more."

Multiple Regression Analyses

Each scale of the MPSI was designated as a dependent measure in a step-wise multiple regression analysis where the independent variables were those described above plus a number of variables which measured the values of the women. The values of the women were obtained by asking each to "Indicate how important these activities are to you now." The activities were (a) being a homemaker, (b) taking part in church, club, and other community activities, (c) being a companion to my husband, (d) helping my or my husband's parents, (e) being a sexual partner, (f) having a paying job, (g) helping my children who are not living at home. Importance was determined by the selection of one point on the following Likert-like Scale:

N / A	0	1	2	3	4
Not Applicable to me	Not Very Important		Neither Important or Unimportant		Very Important

The 42 independent variables used in the step-wise multiple regression included the 22 symptom variables--if present then assign a value of one, if absent then assign a value of zero--and the following 20 variables: age, time since last menstruation, Menopause Index, time since last visit to the doctor, general rating of health status, number of medications taken, regularity of menstruation (zero if regular, one if irregular), painfulness of menstruation (zero if not painful, one if slightly painful, two if painful), moodiness prior to menstruation (zero if no moodier than usual, one if somewhat moodier, two if moodier, three if very moody), frequency of sexual relations (transformed to a monthly frequency), frequency of masturbation or homosexual relations (transformed to a monthly frequency), change in frequency of sexual relations within the last two years (minus one if decrease, zero if no change, one if increase); interest in sexual relations (zero if neither woman nor partner interested, one if partner is more interested, two if both equally interested, three if woman more interested than her partner), and the seven importance/ value variables described in the previous paragraph.

Each step-wise multiple regression was carried out in the following way. The one variable which produced the largest R^2 was selected first. The second variable selected was the one which produced the largest increase in R^2. The third, fourth, etc. variables selected were those which produced next largest increases in R^2. Variables were no longer added to the multiple regression if they failed to increase R^2 by an amount that was significant at the .05 level. Since each step-wise multiple regression included only those women who responded to all 42 variables, the initial analysis was with a much reduced subsample of the total sample. Therefore, after the initial analysis was completed, a second analysis was undertaken in which the step-wise multiple regression was performed using only those variables that significantly increased R^2 after the initial analysis. Again variables which failed to increase R^2 at the .05 level of significance were dropped. If any variables were dropped then a third analysis was undertaken, etc. until no further variables were dropped. This resulted in a maximum number of women being included in the final multiple regression. One outcome of this successive analysis was that as the sample size increased with the dropping of variables, the variables that remained underwent a partial check of their reliability. The final multiple regression prediction equations are presented in Table 2. We see from Table 2 that over 20% of the variance was predicted for Fear of Failure Daydreams and Sexual Daydreams. Table 3 also shows that presence or absence of less than half of the regularly occurring symptoms were salient predictors of various aspects of daydreaming. The presence or absence of six symptoms were involved in the regression equations of three or more daydreaming scales: #8 "lost interest in most things," #9 "feeling unhappy or depressed," #18 "crying spells," #6 "feeling tense, wound up, or irritable," #11 "poor concentration," #4 "heart beating quickly or strongly."

The spurt of daydreaming that was observed in 45-49 year olds by Giambra (1979-80) was not replicated in this larger sample. In general, daydreaming frequency showed a steady drop in each of the half-decades between 40 and 60 years of age. However, when the women in this age group were separated into those who were pre-menopausal, menopausal, and post-menopausal it was observed that menopausal women 45-49 years of age had a much greater daydreaming frequency than any other group. If the 45-49 year old women in the Giambra study were predominately menopausal then this might account for the observed spurt. Unfortunately, the Giambra study also found that men 45-49 years of age also exhibited a large spurt in daydreaming. The attribution of this spurt to menopause seems unlikely.

The most salient outcome of this study was that daydreaming tended to be greater and more prevalent among women who exhibited various psychological and somatic symptoms and who were less physically healthy. The multiple regression analyses showed again and again that both daydreaming propensity and content were most strongly predicted when the symptoms were present. In addition, variables dealing with subjective and objective measures of health were also strongly predictive of daydreaming. The relationship of symptom presence, subjective indications of health, semi-objective indications of health, and attention to body--as indicated by the predictive power of the variable "frequency of masturbation/homosexual relations"-- to increased daydreaming of all types suggests that a self orientation or an introspective preoccupation and daydreaming are connected. The current concerns position of Psychologist Eric Klinger places the symptom presence and health status, as well as the sexual appetite indicated by masturbation/homosexual relations, prior to the increased daydreaming. Thus the current concerns of health and sexual tension would be the source of daydreams dealing with fear of failure and with sexual activity. Indeed, the scales Fear of Failure Daydreams and Sexual Daydreams were the two most predictable of the 13 scales of the MPSI. In a study of the relation between daydreaming and depression in 17-23 year olds, Giambra and Traynor (1978) found that the symptoms used in self-rating depression measures were most highly correlated with Fear of Failure Daydreams scale values. Self-rating depression instruments are essentially symptom check lists; an important corroboration was that the presence of those symptoms also resulted in generally increased daydreaming as well as daydreaming specifically dealing with fear of failure. Many of the symptoms included in the depression self-rating measures were like those obtained in this study's Informational Questionnaire. Thus in 17-23 year olds and in the 40 to 60 year old women of this study the relation between symptom presence and daydreaming was observed.

Table 1

Percentage of Women Showing Each Symptom and Significant (p < .05) Symptom (Present or Absent) Effects on the Scales of the MPSI for the Total Sample and the Married and No Problems Subsamples

Symptom		Percentage with a Symptom			Scales						
					Fear						
No.	Description	Total	Married	No Prob.	Fail.	Guilt	Biz-Imp.	Absorp-tion	Hos-tile	Hall.-Vivid.	Her-oic
9	Feeling unhappy or depressed	27.0	27.5	22.9	TMN	TMN	TM	TMN	TM	TM	TM
6	Feeling tense, wound up, or irritable	48.0	49.5	47.4	TM	TM	TMN	TMN	TMN	TMN	T
8	Lost interest in most things	6.0	5.2	3.5	TM	TM	TM	TM	TM	TM	TM
18	Crying spells	8.3	8.0	7.6	TM	TM	TM	TMN	T	TM	TM
11	Poor concentration	15.9	15.7	12.7	TMN	TMN	TM	TMN	TM	TM	T
16	Aches in back of neck and skull	15.5	15.7	12.2	TM	TM	TM	TM	TM	TM	TM
14	Excitable	13.4	14.2	9.6	TMN	TMN	TMN	TMN	M	TM	
5	Worrying needlessly	33.5	34.5	28.0	TMN	TM	TM	TM	TMN	TM	TM
21	Feelings of suffocation	2.7	3.0	2.0	TM	TM	TM	TM	TM	TM	TM
10	Attacks of panic	6.7	7.2	3.5	TMN	TMN	TM	TMN	TM		TM
2	Feeling tired or lacking in energy	46.1	46.7	44.3	TM	TM	TM	TMN	TM		TMN
12	Have unusual trouble sleeping	16.1	17.7	18.3	TMN	TM	TMN	TM	TM	TMN	
4	Heart beating quickly or strongly	13.2	14.0	12.2	TMN	TMN	TM	M	TMN	TM	TM
7	Part of body feels numb or tingling	16.7	16.0	12.7	TM	TM	T	T	TM	M	TMN
22	Excessive sweating	12.7	13.7	13.2	TMN	TMN	TMN	TMN	TMN	TN	T
3	Pressure or tightness in head	13.6	14.2	13.2	TM	TM		TM	TM		
15	Weight gain	30.8	32.0	30.6	TMN	TM	TMN			TMN	TM
19	Headaches	22.4	24.0	22.9		TM		TM	TM		
1	Feeling dizzy or faint	11.9	12.2	9.6	TM	T		TM	T	TM	
13	Hot flashes	31.6	34.0	26.5	TM	T	TM	M	T	TM	
20	Blind spots before eyes	2.5	3.0	3.0		TM				TM	
17	Cold hands and feet	10.9	11.5	11.2	TM	M					

Note: Entries indicate significant effect for total (T) sample, married (M) subsample, no-problems (N) subsample.

Table 1 Cont'd.

No.	Symptom Description	Achievement	Frequency	Sexual	Scales Prob. Solv.	Self Rev.	Accept. Day.
9	Feeling unhappy or depressed	TM	TM	TM	TM	TM	
6	Feeling tense, wound up, or irritable	TM	TM	TM	TM	TM	
8	Lost interest in most things	TM	TM	TM	T		T
18	Crying spells		TM	TM	TM	TM	
11	Poor concentration	TM	TM	TM	T		M
16	Aches in back of neck and skull	M	TM	TM	N		
14	Excitable	TM	.	TM	TM	TM	TM
5	Worrying needlessly	TMN	TM				
21	Feelings of suffocation	TM	M				
10	Attacks of panic	TM					TM
2	Feeling tired or lacking in energy	M	TM				
12	Have unusual trouble sleeping	T	TM				N
4	Heart beating quickly or strongly	M					
7	Part of body feels numb or tingling	TM	M				
22	Excessive sweating	N	M				
3	Pressure or tightness in head		TM			MN	
15	Weight gain						
19	Headaches		TM		N		
1	Feeling dizzy or faint	T	T				
13	Hot flashes						
20	Blind spots before eyes	TM					
17	Cold hands and feet	TM					

Table 2

Final Multiple Regression Prediction Equations For Each Scale of the MPSI For the Total Sample in Order From Largest to Smallest R^2

R^2	n	Multiple Regression Prediction Equations
.2182	462	Fear of Failure Daydreams = 1.49 + (.44 X General Health Rating) + (0.97 X Symptom #9) + (1.85 X Symptom #8) + (1.18 X Symptom #11) + (1.44 X Symptom #14) + (1.05 X Symptom #4)
.2040	388	Sexual Daydream = 21.00 - (0.32 X Age) + (1.54 X Freq. of Masturbation/Homosexual Relations) + (1.23 X Interest in Sexual Relations) + (2.78 X Symptom #6)
.1531	445	Hostile Daydreams = 3.25 - (0.33 X Importance of Community Activities) - (0.39 X Last Visit to Doctor, Coded[1]) + (1.40 X Symptom #6) + (2.41 X Symptom #8) + (1.19 X Symptom #11) + (1.75 X Symptom #4)
.1428	440	Absorption in Daydreaming = 2.86 + (0.38 X Freq. of Masturbation/Homosexual Relations) + (2.14 X Symptom #9) + (2.28 X Symptom #18) + (1.02 X Symptom #19)
.1319	460	Guilt Daydreams = 1.90 - (0.28 X Last Visit to Doctor, Coded[1]) + (2.11 X Symptom #8) + (1.33 X Symptom #14) + (1.32 X Symptom #4)
.1050	440	Bizarre-Improbable Daydreams = 3.34 + (0.51 X Pre-Menstrual Moodiness Rating) + (1.48 X Symptom #6) + (2.27 X Symptom #8) + (1.35 X Symptom #11)
.0926	427	Problem Solving Daydreams = 6.37 - (0.83 X Last Visit to Doctor, Coded[1]) + (0.54 X Freq. of Masturbation/Homosexual Relations) + (3.41 X Symptom #18)
.0925	433	Daydreaming Frequency = 6.25 + (0.55 X Freq. of Masturbation/Homosexual Relations) + (1.49 X Symptom #9) + (2.51 X Symptom #18) + (1.37 X Symptom #16)
.0709	365	Achievement-Oriented Daydreams = 1.80 + (0.60 X Pre-Menstrual Moodiness Rating) + (0.39 X Importance of Having a Paying Job) + (2.93 X Symptom #8)
.0683	472	Hallucinatory-Vividness of Daydreams = 1.91 + (1.73 X Symptom #9) + (1.14 X Symptom #16)
.0683	422	Self-Revelation = 12.63 - (0.44 X Last Visit to Doctor, Coded[1]) + (0.45 X Freq. of Masturbation/Homosexual Relations) + (3.17 X Symptom #18)
.0582	464	Heroic Daydreams = 1.73 - (0.22 X Last Visit to Doctor, Coded[1]) + (1.45 X Symptom #8) + (1.02 X Symptom #7)
----	---	Acceptance of Daydreaming = (no predictors)

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[1] The time since the last visit to a doctor or specialist was coded as follows: less than 6 months (0), 6-12 months (1), 13-23 months (2), 24-35 months (3), 36-47 months (4), 48-59 months (5), 60-71 months (6), 72 or more months (7).

Significance to Bio-Medical Research and Program of the Institute: The study of daydreaming is fundamentally a study of thought processes. In order to understand fully the thought processes of man, the total spectrum of those processes needs to be examined. In addition, it is important to know how this wide spectrum is affected by aging. Thus the study of daydreaming in adults, along with other variables, such as differences in age, socio-economic status, attitudes, etc., may help us understand the fundamental processes which underlie all these behaviors.

Proposed Course of Project

Since many of the present experiments are early in the data collection stage and since available resources in terms of personnel and equipment are being fully utilized, no additional studies are being planned for the next fiscal year.

Publication:

Giambra, L.M. Daydreaming, attentional processes, and curiosity in White Americans: Religious, educational, economic, and residency influences for a life span sample. *Journal of Clinical Psychology*, 1981, *37*, 262-275.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00063-14 LBS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Learned Modification of Visceral Function in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Joseph	Senior Staff Fellow	LBS, GRC, NIA
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	J. Thompson	Senior Staff Fellow	LNS, GRC, NIA
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COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Psychophysiology

INSTITUTE AND LOCATION
GRC, NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.5	4.4	5.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate the role of the central nervous system in behavior. In some experiments monkeys (Macaca mulatta) are used to examine the extent to which reflex adjustments of the cardiovascular system can be modified by instrumental cardiovascular conditioning (ICC). Thus far, three procedures have been utilized. In the first, electrical stimulation is administered to localized CNS cardioacceleratory-pressor or cardiodeceleratory-depressor sites, e.g., hypothalamus, and analyses are made to determine if the animal can learn to alter their effects; in a second procedure animals are trained to perform on a food-reinforced exercise task and the ability of the animal to control heart rate during combined exercise and ICC sessions is examined, in a third, pharmacological agents which produce pressor or depressor responses are given during ICC and/or during control periods and baroreflex sensitivity is examined. In other experiments we examine age-related changes in pre- and postsynaptic portions of various central neuronal systems of the rat and mouse using behavioral and biochemical analytical methods.

Project Description:

Objectives: A-C. To determine the neural mechanisms involved in cardiovascular conditioning in monkeys.

D-L. To analyze age differences in biochemical and structural characteristics of the brain of the rodent in relation to behavior.

Methods Employed: A. There have been a great number of experiments in which electrical stimulation of the brain (ESB) has been delivered to various CNS structures and subsequent cardiovascular changes examined. However, most of these studies have been carried out in infraprimates. In the few studies which have been done in primates, the animal was anesthetized and unable to interact with its environment. Therefore, there was no chance to observe the possible interrelationships among ESB, cardiovascular changes and behavior of the animal. The present experiments were designed both to examine these relationships and to carry out a thorough mapping of the cardiovascular control areas in the subhuman primate. Three monkeys were operantly conditioned to speed and to slow heart rate (HR) in sessions comprised of a 512 sec baseline period and a 2048 sec testing period. Three types of sessions were examined: "speeding sessions" (F) during which the animal was operantly trained to speed its heart, slowing sessions (S) during which the animal was trained to slow HR, and no feedback (NF) sessions in which the animal was not required to speed or to slow HR. It simply sat in a closed booth throughout a 512 sec baseline period and a 2048 sec "training" period. Information on speeding and slowing was provided by lights. A red light indicated that the animal was to slow HR and a green light indicated the speeding condition. A white light functioned as a reinforcement light to indicate to the animal that it was performing correctly. A 10 ma, .45 sec electric shock was delivered to the tail once/8 sec for incorrect responding. Systolic (SBP) and diastolic (DBP) blood pressure were recorded during these sessions from chronically indwelling catheters in the external iliac arteries of the monkeys. The BP signal was electrically conditioned to permit the measurement of HR. The sessions were divided into 16 segments of 128 sec duration. Following training, an acrylic platform containing holes drilled to pre-specified anterior-posterior and lateral coordinates approximately corresponding to those of the several different brain regions, e.g., posterior hypothalamus was stereotoxically mounted on the monkey's skull and cemented in place under aseptic conditions. Following a 1 week recovery period, the animal was given .5 mg/kg of phencyclidine, i.m., and electrodes were then passed through the skull and lowered by hand until a suitable (see below) cardiovascular effect was obtained. Stimulation was delivered and the cardiovascular changes observed by examining BP and HR tracings on a polygraph. When a suitable area was found, the lowest intensity was chosen that reliably would produce a HR change of 10-20 BPM and comparable BP change. The electrode was then cemented in place. Dose response curves were carried out in the fully awake animal and intensities were adjusted to produce a 10-20 BPM change in HR. Intensities usually ranged from 350 to 1000 ua depending upon the animal and the area being stimulated. Cardiovascular responses were then examined during S, F, and NF sessions carried out as previously described except that each segment and the baselines were shortened to 64 sec and 512 sec respectively. Stimulation for each session was delivered on alternate segments. Only one area was studied within a set of 3 sessions (S, F, NF). At present only accelerator-pressor areas have been examined. Two areas were examined/day on alternate days; data were analyzed by examining changes from baseline during each stimulation and non stimulation segment In a subsequent experiment two monkeys were trained to control HR and electrodes were implanted as previously described. Following determinations of the areas which the animals were and were not able to bring under control, pharmacologic analyses of the relative contributions of the parasympathetic and sympathetic components of the autonomic nervous system in mediating ESB effects ware carried out. Either the peripheral muscarine, cholinergic antagonist, methylatropine (MA) or the B adrenergic antagonist propranolol (PROP) were administered prior to baseline and subsequent NF sessions. Drug administration was carried out on alternate days. Saline was given on interpolated days. All MA tests were completed before PROP tests were begun. Following these tests other central sites were explored in these animals. These studies are continuing.

B. In a related series of experiments we have been examining the constraints on physical exercise produced by instrumental cardiac conditioning. In these experiments a monkey was trained to pull a lever attached to a weight box containing either 4 or 6 kg, depending upon the size of the animal, in order to receive a 300 mg food pellet. The monkey was trained to receive a reinforcement on a fixed ratio schedule of reinforcement: 1 pellet for each 12 lever pulls (FR-12). HR, SBP, DBP, and number of lever pulls and O_2 consumption were measured during each session. Once stable exercise rates were achieved, each animal was trained as previously described (A) to slow its HR. HR, SBP, DBP, and O_2 consumption were measured during these sessions as well. Once the monkeys were slowing HR reliably, exercise and HR slowing sessions were combined so that the monkey had to pull the lever and slow its HR. The cardiovascular parameters previously mentioned were recorded.

C. There is an abundance of evidence that the cardiovascular (CV) system is modulated by neurogenic reflexes. However, the emphasis on reflex modulation of CV function has fostered a lack of appreciation of the extent to which behavioral events also can modulate CV function and even can inhibit reflexes. One example of this reflex inhibition is the case of baroreceptor inhibition during exercise. It is the purpose of this series of experiments to show that baroreceptor sensitivity also can be modified in experiments in which animals are operantly conditioned to slow or speed HR. Three monkeys were trained to control HR as previously described. After they were fully trained, each animal had a catheter chronically implanted in the cephalic vein with its tip in the subclavian vein. This cannula permitted us to inject phenylephrine or nitroglycerin to raise and to lower BP respectively during F, S, or NF sessions. HR, SBP and DBP were monitored on a beat-to-beat basis as previously described. Each animal received 60 baroreceptor tests with each drug for a total of 120 tests. Twenty tests were given under each of the three experimental conditions (S, F, NF). All 60 of the tests with phenylephrine were completed before any of the nitroglycerine tests were initiated. Baroreceptor sensitivity was determined for each drug by measuring the linear rate of change of heart period (in ms.) during the drug induced blood pressure rise. For phenylephrine the sensitivity (ms/mm Hg) was measured over 6 to 8 consecutive heart beats; for nitroglycerin sensitivity was measured over 12 to 15 consecutive heart beats.

D. It has been reported previously that a surgical lesion in the substantia nigra of young rats results in a substantial decline in the level of dopamine (DA) in the caudate nucleus on the lesioned side. The caudate nucleus has been shown to be very important in the control of movement. An imbalance in the neurotransmitters between the lesioned and unlesioned nigrostriatal pathways produces a directional dominance in an animal's movements such that after injection of certain drugs such as apomorphine (a DA receptor agonist) or amphetamine (which promotes the release of DA) animals can be induced to turn in circles. These rotations can be accurately measured with a device called a rotometer. Numerous studies have shown that: (a) amount of depletion; (b) DA receptor activity; (c) development of denervation receptor hypersensitivity; and (d) presynaptic release events can be gauged by the strength and directionality of the rotational behavior. This behavior seems to be almost exclusively modulated by the nigrostriatal system and by dopaminergic-cholinergic interactions within this system. By using this technique in conjunction with several biochemical assessments, we are exploring, (a) the locus of the synaptic deficits that appear with senescence and the methods by which these deficits can be reduced, (b) factors which alter striatal DA receptor number in the aged rodent, (c) the particular DA receptor type that is most closely related to motor behavior in the rodent. Detailed methods are given below for examining the latter two (b and c). In this section we will concentrate on the first (a) of these factors.

In a previous experiment we reported that senescent animals unilaterally lesioned in the left substantia nigra and pretreated with L-DOPA prior to amphetamine administration do not show increases in rotational behavioral strength. This deficit appears not to be the result of losses of dopa decarboxylase or tyrosine hydroxylase in the senescent animal. It is also not the result of lowered activity DA levels in these senescent animals. In the present studies we explored the striatal DA uptake and release mechanisms using two procedures: 1. Unlesioned male rats (3 old and 4 young) were used to assess the amount of intraperipheral injected L-DOPA actually entering the striatum. These rats were injected with 5 mg/kg [^{3}H] L-DOPA (New England Nuclear Co. 11.4 Ci/male. One hour later they were killed by decapitation and the striata were rapidly removed. After weighing, each striatum was dissolved in Solulene at 50°C overnight and radioactivity was determined using a Dimilune scintillation cocktail (Packard). 2. Male Wistar rats 7, 12, 24 mo of age were decapitated and striata rapidly removed and washed in Evile's salts solution. Each half of a striatum was sliced on a Sorvall Tissue Chopper (200 mm sections). One-half of a striatum was placed in 2 ml Earle's salts plus abscorbic acid (0.5 mg/ml). After incubation in [^{3}H] DA the amount of [^{3}H] DA accumulated in the cells released by KCl and amphetamine was determined.

Also examined in other experiments was basal, DA stimulated adenylate activity in 3, 6, 12 and 24 mo old rats. Striata were dissected from the brains of animals 3, 6, 12 and 24 month old and homogenized in 1.0 ml of 10 mM Tris maleate pH 7.5 using a glass homogenizer with a motor driven Teflon pestle. Adenylate cyclase activity was assayed on 10 ul aliquots of homogenate in tubes in a final volume of 50 ul, 22 mM Tris maleate pH 7.5, 2mM $MgCl_2$, 1 mM A+P, 1 uCi [^{32}p] α ATP, 1mM cyclic Amp, 10,000-20,000 CPM [^{3}H]-cyclic AMP 0.4 mM isobutylmethylxanthine 29 mM creatine phosphate, 25 ug creatine phosphakinase and 10 mM pargyline. Dopamine (1-100 uM), EGTA (1 mm) and GMPP (NH)p (10 uM) were included in various combinations indicated. The reaction was initiated by the addition of homogenate carried out for 5 min at 30° and stopped by the addition of 100 ul of a solution of 2% SDS 4 mM ATP, 1.4 mM cyclic AMP. The $[^{32}P]$- cyclic AMP was isolated, counted in a Packard 2650 scintillation spectrometer corrected for recovery using the $[^{3}H]$- cyclic AMP monitor. Protein was determined by the Lowry method.

E. In a second series of experiments we examined the factors which alter striatal DA receptor sensitivity in the aged rodent by using 6-OHDA lesions or chronic haloperidol administration. Additionally, we examined, through correlational procedures the DA receptor type most closely associated with motor behavioral activity (rotation). Alterations in regulation of the DA receptor following 6-OHDA lesions were examined by unilaterally lesioning the young and old rats. Following a recovery period (7-10 days), the lesioned animals were given an initial test with amphetamine and nonturners were discarded. After a 30 day waiting period, the animals were tested for the development of striatal DA receptor supersensitivity with the DA agonist lergotril. Three weeks later they were sacrificed and DA receptor activity was analyzed by measuring the amount of $[^{3}H]$ spiroperidol binding and DA stimulated adenylate cyclase activity in the striata from the lesioned and unlesioned hemispheres. Reaction to chronic haloperidol injections was measured in young (6 mo) and old (24 mo) animals given haloperidol 2 mg/day/21 days.

F. In a third series of experiments graded doses of DA releasing agents (e.g., amphetamine 0, 2.5, 5.0, 7.0, 10.0) and agonists (dopamine (0, 5, 25, 50 ug)) were injected into the striata through chronically implanted cannulas in rats unilaterally lesioned in the left substantia nigra. Rotational behavior was examined following these injections.

G. Finally, in a fourth series of experiments, nialamide, a monoamine oxidase inhibitor, was peripherally administered at various doses, and several different stereotypic behaviors postulated to be related to striatal DA activity (e.g., gnawing, sniffing, rearing, grooming) were examined. Additionally, open field locomotor activity, and emotionality also were assessed in order to determine if there were any age-related differences in these behaviors following nialamide.

H. Parametric studies were conducted to determine age differences in the performance of male C57BL/6J and A/J mice in a battery of motor tests, including grip strength, tightrope tasks, stationary and rotating rod tasks, and exploratory and runwheel activity. These animals were sacrificed and bioassays were made to determine levels of activity for tyrosine hydroxylase (TH), glutamic acid decarboxylase (GAD), and choline acetyltransferase (CAT) in the cerebral cortex, hippocampus, striatum, and cerebellum.

I. Studies were conducted to determine age differences in the performance of male C57BL/6J and A/J mice in step-through passive avoidance conditioning. From lifespan representative age groups mice are placed in one side of a two-sided compartment. Lights are activated in that side and a door is opened to a dimly lighted compartment. When the mouse enters the adjacent chamber, the door is closed and electric foot shock is delivered. The animal is removed and tested for retention of this learning at various intervals.

Male C57BL/6J mice have also been tested in operant shock escape tasks. The animal is placed into operant chambers containing two levers. Foot shock is delivered on a variable interval 30-sec schedule. The mouse must learn to escape shock by pressing the reward bar. The procedure steps through this FR-1 (fixed ratio) schedule to a FR-3 schedule, requiring three bar presses to terminate shock. In subsequent sessions the mice are trained in a reversal task requiring responses on the second bar to terminate the shock, with responses on the first bar being inoperative.

Young and aged male C57BL/6J are also being tested in a complex 14-unit multiple T-maze, with water as behavioral reinforcement. Mice are first adapted to a 22-hr. fluid deprivation schedule and trained to run for water reward in a straight alley. The mice are then given one daily trial in the complex maze over a period of 28 days with entries into arms deviating from the path to the goal box counted as errors.

Young and aged female Wistar rats have been tested in radial maze performance. The animals are first reduced to 75% of baseline body weight for young and aged groups respectively and then trained to run in a straight alley for food reward (sweetened milk). In one daily trial, the animals are then placed into the center of the radial maze and allowed to freely explore among multiple arms radiating from the center. A food reward is placed at the end of each arm; thus, the optimal strategy is to enter all arms of the maze without re-entering arms, which constitutes an error. This paradigm was designed as a test of the rat's ability to form "cognitive maps" of its enviroment and, as such, employed elements of short--term memory to a much greater degree then in the 14-unit T-maze which stressed long-term memory to a greater degree. At the end of training, rats were sacrificed and bioassays were made to determine the activity of TH, CAT and GAD in the hippocampi and cerebral cortices of the rats.

J. Animals were subjected to various nutritional and exercise regimens at different stages in their development to determine the effects of these treatments upon lifespan, to find developmental correlates of lifespan, and to assess behavioral vigor at advanced ages. Nutritional manipulations include level of dietary protein (4%, 26%, or 48%) and schedule of restricted feeding (every-other-day). Subjects include male and female Wistar rats, several strains of inbred and hybrid mice, and obese mutants. One study also compared aged virgin and retired breeders from the DBA/2J mouse strain. Behavioral and physiological assessments are made of the effects of the treatments, including tests of exploratory and runwheel activity, grip strength, tightrope, stationary and rotating rods, passive avoidance and maze learning, food intake, metabolic rate, and body temperature.

K. Numerous studies have shown that the level of general activity declines as a function of age. This decline may reflect a decrease in motivational level in senescence. Thus, an experiment in which motivational levels could be enhanced through highly rewarding reinforcements could function to decrease these declines in activity. Intracranial self-stimulation (SS) has been an appropriate model for these purposes, because it is related to activation of the basic motivational mechanisms and the stimulation in lateral showed hypothalamus can be highly rewarding.

Two age groups of male mice - 12-14 and 28-30 month were studied. Under pentobarbital anesthesia concentric electrodes were implanted in the field of the medial forebrain bundle on the level + 2 mm from the interauricular line. A special technique was developed for the long term fixation of electrodes and connectors to the skull. After recovery, the animals were placed in a Skinner box and each bar press resulted in delivery of 0.5 sec train of square waves (frequency -100/sec, duration - 0.5 msec. The intensity was adjusted to effect for each animal and ranged from 50 to 500 ua.

L. Literature concerned with age changes in thermoregulation is inconsistent. One question has been generated by some reports that indicate that old animals (rats, mice) have a lower rectal temperature then young at room temperature. However, other reports are at variance with these wherein no differences in body temperature between young and old mice were found.

A second question concerned with thermoregulation and aging is that of maintenance of body temperature in a cold environment. One report has found that mortality rate on exposure to cold was much higher for old mice then for mature young, and others demonstrated significant differences in ability to maintain temperature during short term exposure to cold between adult and old mice. Other reports, however, did not find age difference for rats under similar conditions. Thus, this project is being carried out to clarify these inconsistencies.

In order to clarify question 1, rectal temperature of mice was measured for different age groups (3,6,9,12,15,20,24,28,30,32 mo) from the GRC colony. To answer question 2, mice were food deprived for 20 hrs and placed in small plexiglass restraining cylinders which were placed in a room where the temperature was maintained at 10°C. They were left there for 3 hrs and body temperature was monitored.

Major Findings: A. The effects of ESB on HR and BP during S, F, and NF were analyzed in three monkeys from 4 brain regions: (I) lenticular nucleus, globus pallidus, ventromedial tegmentum; (II) anterior amd/or dorsomedial hypothalus; (III) posterior or lateral hypothalamus; (IV) subthalamic nucleus. A fourth monkey died before completion of the study and only 2 brain areas were examined in it (I, III). Results showed that there were significant interactions between experimental conditions (S,F,NF) and ESB when changes in HR (ΔHR), SBP, and DBP from baseline to each stimulation (ST) and non stimulation ($\overline{\text{ST}}$) segment was analyzed using a 2 (ST; $\overline{\text{ST}}$) by a 3 (S,F, NF) mixed model analysis of variance: (a) For each animal during slow sessions Δ HR was significantly attenuated when ESB was given in regions I or III but not when regions II or IV were stimulated; (b) During speeding Δ HR either was enhanced or was similar to Δ HR during NF but this varied among animals and regions; (c) BP was not reliably altered during ST--i.e., there were no consistent effects across animals. Thus, these experiments show that animals can alter the effect of ESB through behavioral conditioning and that the degree of alteration can be very great. Results for a typical animal are shown below for Δ HR in beats/min. Note that the scores in this table are changes from baseline. Thus, ST effects are superimposed on pre-existing performance levels. Compare ST and $\overline{\text{ST}}$ during S or F with ST and $\overline{\text{ST}}$ during NF.

	S		NF		F	
REGION	ST	$\overline{\text{ST}}$	ST	$\overline{\text{ST}}$	ST	$\overline{\text{ST}}$
I	-32.3	-33.8	1.1	-7.3	20.5	13.5
II	-3.9	-21.2	51.1	29.8	42.0	26.4
III	-14.1	-14.1	-1.0	-10.8	22.5	8.6
IV	11.0	-10.3	23.0	9.6	29.8	14.0

In subsequent experiments in two additional monkeys, we again found that ESB to anterior placements in the hypothalamus (at AP 12.0 anterior to ear-bar zero in the dorsametral hypothalamus and the region of the supraoptic nucleus) cannot be controlled by the monkeys. ESB to placements in the region of the sub-thalamic nucleus also could not be controlled. One monkey could control ESB delivered to the region of the caudate nucleus. In the other monkey the placement at this anterior level was more medial and was located in the anterior hypothalamus. In these animals either methylatropine or propranolol essentially eliminated the increases in ESB induced Δ HR in all areas that we have examined thus far, e.g., animal 4 sub-thalamic nucleus control Δ HR 18.3 ± 2.6 methylatropine -6.7 ± 1.2; propranolol, 18.7 ± 2.2 -5.3 ± 1.3.

These data show that an animal can significantly attenuate its HR responses to stimulation to the posterior hypothalamus and striatum during conditions where a negative reinforcement contingency is placed on HR slowing. But it is not able to attenuate HR responses when stimulation occurs in the anterior hypothalamus or subthalamic regions.

Other investigators have shown that stimulation in the anterior hypothalamus and subthalamic nucleus evokes affective as well as cardiovascular responses. For example, it has been shown that lesions in areas in the baboon corresponding to our anterior hypothalamic sites create deficits in an animal's ability to generate a conditioned emotional response, i.e., ΔHR or ΔBP, to a conditioned stimulus that was given prior to the administration of an electric shock. Lesions outside this zone did not have similar effects. It is possible in the present experiments, that affective responses elicited by stimulation in these areas override the reinforcement strength of shock avoidance. However, the striatal or posterior hypothalamic stimulations can be overridden because they are associated with cardiovascular effects only. Posterior hypothalamic stimulation may involve only the outputs from the anterior hypothalamic area. The subthalamic nucleus is a complex area that contains motor as well as cardiovascular functions and may receive selective input from the anterior hypothalamus and striatum. We are presently carrying out research to examine imputs to these areas. Nevertheless, one conclusion is clear--CNS-autonomic interactions in the unanesthesized animal are not invariant and can be modified by training. Differences in response to ESB suggest that there are a variety of mechanisms operating within the brain that mediate plasticity such as that seen here. HR responses, even in the face of ESB, can be different depending on the environmental contingencies and the demands made on the animal.

B. This project has received some setbacks as a result of loss of trained monkeys through illness and/or catheter failure and equipment failure. It is continuing with additional animals.

C. The tables below show the major findings of this study, which is now completed. The three animals tested responded with identical patterns. During slowing, baroreceptor sensitivity was similar to the control condition when phenylephrine was injected, but was significantly inhibited during nitroglycerin injection (Table 1, II). During speeding baroreceptor sensitivity was significantly inhibited during both drug conditions (Table 1, II). Since SBP reactivity was not attenuated during either of the experimental conditions relative to the control condition (Table 2), the experimental effect clearly influenced the cardiac response.

Table 1

Cardiovascular adjustments to baroreceptor stimulation during operant cardiac conditioning (ms/mmHg)

I. Nitroglycerin

Animal	Doseage	Condition Slow	 Speed	 Control	F-ratio‡
1	100 ug/kg	1.15	0.96	1.81	3.85*
2	200 ug/kg	0.23	1.28	2.56	5.90**
3	50 ug/kg	0.68	0.97	1.13	2.83

II. Phenylephrine

Animal	Doseage	Condition Slow	 Speed	 Control	F-ratio!
1	20 ug/kg	1.52	0.91	1.69	6.10**
2	25 ug/kg	2.45	0.21	2.64	11.07**
3	10 ug/kg	4.91	1.93	4.18	4.18**

‡ df = 2,57 for animal 1 and 2
df = 2,27 for animal 3
*p<.05
**p<.01
!df = 2,57 for all animals

Table 2

Systolic blood pressure ranges during baroreceptor sensitivity testing.

I. Nitroglycerin

Animal	Condition Slow	 Speed	 Control	F-ratio‡
1	-24.6	-29.6	-23.4	7.41**
2	-19.3	-27.2	-17.7	6.13**
3	-35.3	-34.7	-40.6	1.97

II. Phenylephrine

Animal	Condition Slow	 Speed	 Control	F-ratio!
1	38.4	37.2	32.7	3.17*
2	24.4	21.2	23.0	0.93
3	42.7	37.0	47.2	5.81**

‡df = 2,57; 2,27 for animal 3; *p<.05; **p<.01
!df - 2,57 for all animals

The results of these experiments show that in both cases where the animals had to override the baroreceptor reflexes in order to escape electric shock they did so. It is evident from these findings that reflex adjustments can be attenuated reliably by operant contingencies. Thus, it would seem that conditions which threaten the well being of the animal will produce behaviorally mediated adjustments in CV reflexes.

D-G. The studies reported in this program are inter-related and will be reported together. They have concentrated on trying to determine the striatal locus (presynaptic or postsynaptic or both) of the age-related deficits previously observed in rotational behavior following amphetamine administration. In the intitial experiment L-DOPA was administered prior to amphetamine in order to see if a precursor of DA would raise DA levels in the old animal and subsequently enhance rotational behavioral response strength. Results showed that young rats increased their left/right turn ratios by 50% while old rats showed a 23% decline in responding. No changes were seen in dopa decarboxylase and hydroxylase activity. Subsequent experiments have shown that the amount of [^{3}H] L-DOPA entering the striatum was actually higher in the senescent rat than in the young rat (old 1065 ± 153 dpm/mg tissue; young 510 ± 70; t(12) 3.19 $p<.01$). *In vitro* uptake of DA and release of DA following KCl or amphetamine administration onto striatal slices from young and old rats also show no alterations with senescence [Uptake, age x time analysis of variance, F(6,45) = 1.66 $p>.05$; Release to KCl, 24.1% (7 mo old animal) 23.2% (12 mo old animal), 21.9% (24 mo old animal) all $t<1$; ED50 of 2.5×10^{-3}m was found for all ages for amphetamine]. An analysis of variance showed no difference in peak release of DA by amphetamine across ages (F(2,21) $p>.05$). Thus, it appears that the pre-synaptic "machinery" is functioning well in the senescent animal. When receptor mechanisms are examined, however, the story may be different. We had already shown a 33% loss in [^{3}H] haloperidol specific binding in the striata of the senescent rat. Now we examined basal and DA stimulated striatal adenylate cyclase activity. This activity has been suggested as the marker for another type of DA receptor. In further experiments we have shown that basal, striatal adenylate cyclase activity declines by about 20% from 3 to 24 mo in both males and females. But the largest differences in males occur during the maturational phase (after 3 mo). No differences are seen after 6 mo of age. A similar finding was seen in males for 100 uM DA stimulation of adenylate cyclase activity. Females showed a similar pattern of decline but the basal adenylate cyclase activity was approximately 50% higher in females compared to males of all ages.

Interestingly, the old animal can exhibit some plasticity in the face of such deficits, since one set of experiments recently completed has shown that similar denervation hypersensitivity concomitant with striatal DA receptor proliferation following lesions of the substantia nigra takes place in both old and young animals. The lesions in these animals were all chemically (6-OHDA) produced in the left substantia nigra. Therefore, following the 30 day recovery period, the animals turned contralaterally to the lesion (right) following lergotril administration. A behavioral index of right/left turns was computed. No age-related differences in this index were seen. Three weeks after these tests the animals were sacrificed and ratios of adenyl cyclase activity and [^{3}H] spiroperidol specific binding in the lesioned left striatum relative to the unlesioned right striatum were computed (L/R). These values are given in Table 1.

Table 1
Left/Right Striatal Ratios for $[^3H]$ Spiroperidol Binding and DA Stimulated Adenylate Cyclase Activity

	N	$[^3H]$ Spiroperidol Specific Binding	100 uM AC	5 uM AC	1 uM AC
*Mature males	6	1.37 ± 0.12	1.28 ± 0.05	1.78 ± 0.18	2.48 ± 0.60
Senescent male	6	1.48 ± 0.07	1.48 ± 0.07	1.78 ± 0.22	2.05 ± 0.18
Mature females	4	1.51 ± 0.26	1.25 ± 0.07	1.56 ± 0.27	2.00 ± 0.38
Senescent females	5	1.44 ± 0.19	1.14 ± 0.14	1.42 ± 0.34	2.00 ± 0.63

*Values represent means ± standard error for numbers of experiment indicated.
+The $[^3H]$ spiroperidol concentration which gave the highest amount of specific binding was used to calculate these ratios.

As can be seen there are no systematic age or sex differences in these ratios, indicating that old animals had not lost the ability to proliferate receptors following lesions--"Plasticity in the face of a deficit." We also showed in this same experiment that rotational behavior is more highly correlated with DA receptors linked to $[^3H]$ spiroperidol specific binding than to those linked to adenylate cyclase.

Table 2
Partial r Values Denoting Relationships
(a) Rotation (T) and $[^3H]$-Spiroperidol Specific Binding (SP)
(b) Rotation and Adenylate Cyclase Stimulation (uM DA)

	df (N-5)	SP	1 uM DA	5 uM DA	100 uM DA
Males T	7	.94+	-.74*	-.57	-.31
Females T	4	.68	-.63	-.47	-.06
Young T	5	.93***	-.64	-.45	-.30
Old T	6	.75	-.76*	-.63	-.08
Overall T	16	.93+	-.67+	-.49*	-.27

*p<.05; **p<.02; ***p<.001

These findings suggest an important role for the DA receptors linked to $[^3H]$ spiroperidol specific binding in the control of motor behavior. Moreover, it may be the loss of these receptors with senescence that is contributing to the decline in rotational behavioral strength seen in these experiments. No data are available as yet on the production of receptor hypersensitivity through chronic haloperidol injections.

Finally, we are continuing to examine the effect of monoamine oxidase inhibition (with nialamide) on many dopamine-mediated stereotypic behaviors in young and old rats. Early results have shown that nialamide reduced these behaviors (e.g. gnawing, sniffing, locomotor activity) in a dose dependent manner in both age groups. However, more reduction was seen in one behavior, rearing, in the young group following nialamide than in the old group. Rearing is a behavior that is very strongly mediated by dopamine. The fact that it is not depressed following inhibition of monoamine oxidase in the old animal suggests that rearing is so low that it cannot be lowered any further with nialamide administration. At present we cannot explain the paradoxical effect that monoamine oxidase inhibition is having on rearing.

Several possibilities are presently being explored. We are also continuing to examine rotational behavioral effects of direct DA agonist application intrastriatally in young and old animals.

H. The tests demonstrated that aging was associated with declining motor abilities in the strains of mice studied; however, there were many interactions involved between task and strain in the demonstration of age-related performance differences. Age-related decrements were observed in both strains in balance rod and wheel activity tests. Among A/J mice there were age-related decrements observed in grip strength and rotorod tests that were not observed in the C57BL/6J strain. Similarly, among C57BL/6J mice there were age-related decrements observed in tightrope and exploratory activity tests that were not observed in the A/J strain. Strain differences were evident in virtually every task. In general, A/J mice had stronger grip strengths and higher tightrope scores, which indicated superior upper body strength. A/J mice performed more poorly in balance rod and rotorod tasks and demonstrated less exploratory and wheel activity compared to C57BL/6J mice.

In regard to differences in neurochemical activity, many complex changes were noted.

Genotype TH activity was higher in the C57BL/6J strain compared to the A/J strain in all brain regions of all age groups except the striatum at 4 mo, hippocampus at 18 mo and cortex at 24 mo where there were no strain-related differences.

CAT activity was higher in the C57BL/6J mice except at 24 mo, when cerebellar activity was greater in the A/J strain.

Age Striatal TH of both strains was not affected by age. TH activity increased in A/J mice between 4 and 18 mo in the cortex, cerebellum and hippocampus and between 4 and 24 mo in the cortex and cerebellum. TH in C57BL/6J mice increased between 4 and 18 mo in the cortex and cerebellum and between 4 and 24 mo in the cerebellum, but declined in the cortex between 18 and 24 mo.

GAD activity declined between 4 and 24 mo in the A/J striatum and C57BL/6J cortex, and increased between 18 and 24 mo in the A/J hippocampus and between 4 and 24 mo and 18 and 24 mo in both striatum and hippocampus of the C57BL/6J mice.

Between 4 and 18 mo and 4 and 24 mo, CAT activity increased in the cortex of both strains, the A/J cerebellum and the C57BL/6J hippocampus. Between 18 and 24 mo, CAT activity was elevated in the A/J cerebellum and the hippocampus of both strains. Striatal CAT increased in C57BL/6J mice between 4 and 24 mo.

In conclusion, age-associated alterations in activities of neurotransmitter synthetic enzymes vary with the genetic strain under investigation. Increases in enzyme activity may represent age-related changes in affinities for substrates or cofactors. These findings underscore the importance of parallel studies using several inbred strains or hybrid strains in aging research. The functional significance of these neurochemical changes is currently being assessed in a multivariate correlational analysis.

I. Thus far, no reliable age differences have been found in retention of passive avoidance learning or in escape learning or in its reversal learning components. These findings are consistent with many past reports in this laboratory that aged animals do not appear to be handicapped in relatively simple learning tasks. In contrast, in the complex T-maze, aged mice have been found to be impaired in performance in comparison to young mice. A major contribution to this age-related deficit appears to be a greater tendency toward perseverative responding among aged mice. Similarly, aged rats were found to be impaired in performance in the radial maze; however, this deficit was not related to perseverative responding. Performance among aged animals in this learning paradigm was found to be correlated with levels of CAT activity in the hippocampus and GAD activity in the cerebral cortex.

J. Results from this laboratory continue to demonstrate the prolongevity effects of two dietary regimens, low protein and feeding every-other-day, in both rats and mice. Studies have also now demonstrated that these treatments might produce a retardation of age-related behavioral and physiological change. Dietary restricted rats were found to be indistinguishable from young rats in performance in the 14-unit T-maze; whereas, old ad libitum fed animals showed deficient learning. Indices of kidney functioning in aged restricted rats, including urea concentration and creatinine clearance, also were not different from young animals but were superior to aged ad libitum fed animals. In preliminary tests comparing dietarily restricted mice to ad lib controls, restricted animals were found to show greater levels of exploratory activity and superior performance in tests of upper body strength. Analyses of striatal dopamine receptors have shown that 24 mo old rats which have been dietarily restricted since weaning have 50% more receptors than do non-restricted, age peers. These levels are comparable to those of 3-6 mo old animals.

Exercise has been another treatment that has proven to increase lifespan in rats when begun early in development but not late in development. Earlier analyses addressed the hypothesis that this prolongevity effect was related to a retardation of growth rates. Further analysis revealed that, within groups of similarly treated animals, this hypothesis was not supported. In fact, longer lifespan was positively related to higher growth rates during late development. The relationship between lifespan and growth rates has been assessed further in several inbred and hybrid strains of mice. Adult body weight and early growth rates were found to be positively related to lifespan among male mice and negatively related to lifespan among female mice. Among aged mice begun on an exercise schedule at an advanced age (20 mo), there was little improvement in behavioral indices of aging except stronger grip strengths compared to caged controls. Examining the relationship between behavioral and physiological variables late in the lifespan of mice, another study found that hematocrit and collagen tests and exploratory activity and passive avoidance learning were possible predictors of longevity. In another study, aged male retired breeder mice were found to be less active in wheel running cages compared to virgin mice of the same age, which might indicate accelerated aging as a result of a breeding regimen.

K. Rectal temperature was lower in the 30 month age group (36.3 ± 1.1) than all other groups (3 mo, 37.5 ± 0.7; 6 mo, 38.0 ± 0.7; 9 mo, 37.6 ± 0.7, 2 mo, 37.7 ± 0.3; F(1,198) = 3.81 $p<.05$). After 20 hrs food deprivation and 3 hrs in the "cold room," the old mice showed a much greater drop in body temperature than young mice (old, 25.9 ± 3.2°C; young 35.9 ± 1.4°C).

L. Results showed that there were no significant differences in SS of two age groups. Mean frequency of the bar pressing was 1786/h. For adult animals this result was 1847/h and for senescent - 1736/h ($p > 0.1$).

Significance to Bio-medical Research and the Program of the Institute: There is growing evidence that the central nervous system provides a high degree of plasticity for peripheral CV and motor functions. Thus, responses which ordinarily occur in one way under one set of conditions, may occur in another way under another set of conditions. For example animals which can learn to modulate their CV responses during such behaviors as predation or evading or escaping predators should have selective advantages over conspecific animals not so well endowed. Thus, learning is an important process which can operate through the nervous system to modulate peripheral function. A second process which may actually reduce plasticity and response capabilities is aging. Until we understand fully how these processes operate, and under what conditions they will express themselves, we will never be able to explain behavior in normal or diseased individuals, or in subjects of any age. Furthermore, when we understand the principles which underlie neurally mediated plasticity, we should be able to evolve strategies which will enable subjects to compensate, at least in part, for the infirmities associated with the disorders of adult life. For example, the neural systems that we are now studying in the primate projects deal with the integration of somato-motor and cardiovascular responses. It is well-known that both of these systems become less adaptable with age; but what is not known is the extent to which this diminished adaptability can be modified by experience. The experiments involving the interaction of ESB and behavior during ICC, and the experiments concerned with instrumental modification of HR during exercise together with those concerned with alterations in baroreflex sensitivity lend themselves well to delineation of the contribution that experience makes in modifying neural events. Another example of the relevance of this project to the assessment of age-dependent neural changes in plasticity comes from the work on the nigrostriatal system. It is well-known that neuromuscular disorders of late adult life such as Parkinson's disease are associated with various changes in function and structure of the nigrostriatal system. It is our goal to characterize these age-related declines and the extent to which they can be ameliorated.

Proposed Course of the Project: A-B-C. Data collection is continuing on the effects of stimulation of various anterior hypothalamic, thalamic, striatal and posterior hypothalamic pressor, depressor cardioacceleratory and deceleratory areas during the three procedures, i.e., fast, slow and no feedback, in order to further characterize the effects thus far observed. In later experiments attempts will be made to place electrodes more posteriorly in brain stem sites to determine if stimulation of these areas can be altered during ICC. Additionally, efforts will be made to determine the vagal and sympathetic contribution to these changes through the use of various peripheral adrenergic and cholinergic agonists and antagonists. Central neurohormonal characterization of these extralimbic and limbic sites will be made by the central injection of various pharmacologic agents via chronic indwelling cannulae implanted in these areas. The exercise experiments are also progressing. Additional animals will be taught to speed HR during exercise and the combined effect of these conditions will be observed. Cardiac stroke volume as well as the other measures will be made during combined and uncombined conditioning and exercise sessions. In future research we also shall try to determine whether attenuation of the baroreceptor reflex which occurs during operant HR conditioning is due to the modulation at the receptor site, the afferent limb of the reflex or the efferent limb of the reflex.

D-G. So far the project has concentrated on the dopamine system. Further investigations will examine turning behavior after modifications on serotonergic, noradrenergic, and cholinergic systems. We will continue to analyze the effects of intracerebral administration of the test drugs on rotation in young and old animals, and we will continue to look at synaptic events such as turnover in striatal slices from young and old animals.

H. Work will continue to improve the behavioral paradigms including reliability testing (test-retest correlation) and validity (age-related behavioral change). New tests are being developed including swimming and jumping performance.

On the basis of the outcome of the correlational analyses, pharmacological treatments are being planned, including the use of cholinergic and dopaminergic agonists. Pilot studies have already been conducted using dopaminergic agonists to increase wheel activity.

I. We will continue to refine the shock avoidance procedures. Studies are planned to assess age differences in consolidation of memory in these paradigms and to assess the effects of pharmacological intervention upon the consolidation process with specific interest in cholinergic agonists. Studies will continue that use water deprivation as a motivator in maze tasks. At present, animals are being sacrificed to determine possible neurochemical correlates of maze learning and pharmacological treatments will be planned. Studies will also continue in the radial maze paradigm. Plans are being made to modify the procedure to reduce the involvement of visual cues (which might be an age confound) and to increase the involvement of tactile cues. In addition, psychophysiological correlates of performance will be made with special interest in hippocampal theta activity. Pharmacological and electrical stimulation studies are also being considered. Other learning paradigms will be explored including conditioned taste aversions and delayed responding in shock escape learning.

J. Additional assessment will be made to determine behavioral and physiological effects of nutritional manipulations. Collaboration has been established to determine effects on cardiac functioning and brain neurochemical activity and receptor density. Additional testing will be conducted to further analyze developmental and behavioral predictors of lifespan.

K. This study will be continued to establish the significance of the separate factors (age, food deprivation, restriction of mobility and cold stress) on temperature homeostasis.

L. Further studies will be carried out to determine the effects of prolonged self-stimulation in old animals to their resistance to physiological stress.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00064-20 LBS

PERIOD COVERED October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Problem Solving and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIs:	David Arenberg,	Chief, Learning & Problem Solving Section	LBS GRC NIA
	Leonard M. Giambra	Research Psychologist	LBS GRC NIA
Others:	None		

COOPERATING UNITS (if any)

Baltimore City Hospitals

Jan Sinnott, NRSA postdoctoral fellowship (sponsored by David Arenberg)

LAB/BRANCH Gerontology Research Center - LBS

SECTION Learning and Problem Solving Section

INSTITUTE AND LOCATION NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS: 1.7	PROFESSIONAL: .4	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are to; (1) identify reasoning processes; (2) determine how these processes change with age; and (3) develop techniques for reducing age deficits in reasoning performance. Reasoning is studied using problem solving procedures including concept learning and concept identification.

Project Description: Z01 AG 00064-20 LBS

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem-solving procedures in which on-going solution behavior can be observed and quantified. Experiments are designed to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: Of the four current studies, three are long-term aging studies; Experiments IV and V are longitudinal studies, and in Experiment XI each subject solves more than 100 problems and a model is constructed which describes and predicts how (s)he solves a variety of complex concept-learning problems. Experiment XII is a new study to investigate complex logical thinking using both abstract and concrete (realistic) type problems. It is hypothesized that problem content (abstract or realistic) affects age-related performance declines. When an older adult appears to fail a problem requiring formal operations, it may be that the failure is due not to inability to solve the problem but to interpreting the problem differently and solving the interpreted problem. Abstract problems requiring Piagetian formal operations and logically equivalent, realistic problems designed to require the same operations are used. A systematic inquiry after the problem is solved is used to determine how the problem was interpreted. Three abstract problems and three realistic problems (which are logical counterparts) are administered to men and women between 18 and 90 years of age.

Major Findings: Data collection continues in Experiment IV, a longitudinal study of logical problem solving, to obtain repeat performance for those men who solved comparable problems six years previously. Data collection continues in Experiment V, a longitudinal study of concept problem solving, to obtain both first-time and six-year repeat performance for men and first-time performance for women. No analysis of data was made in either study this year. Model construction continues in Experiment XI.

Experiment XII is the new study of abstract and logically comparable realistic problems. Problems were constructed and data collection was initiated.

Significance to Bio-Medical Research and the Program of the Institute: Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed to obtain quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of response which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate the pervasiveness of reasoning deficits with age, whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: Data collection for the longitudinal study of concept problem solving (Experiment V) will continue for both men and women. Data collection for Experiment IV, another longitudinal study of reasoning performance, will also continue. Models which describe and predict how an individual solves concept-learning problems continue to be constructed for participants in Experiment XI. Data collection will be completed for Experiment XII; data will be scored, analyzed, and summarized by Dr. Sinnott before her NSRA fellowship terminates (during FY 82).

Publications:

Giambra, L.M., and Arenberg, D. Problem solving, concept learning, and aging. In L. Poon (Ed.): *Aging in the 1980's: Selected contemporary issues in the psychology of aging*. Washington, American Psychological Ass'n., 1980, pp. 253-259.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00065-21 LBS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Verbal Learning and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Arenberg	Chief, Learning & Problem Solving Section	LBS GRC NIA

Others: None

COOPERATING UNITS (if any)

	Baltimore City Hospitals
Elizabeth A. Robertson-Tchabo	University of Maryland
Jan Sinnott	NRSA postdoctoral fellow (sponsored by D. Arenberg)

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning and Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS: 2.3	PROFESSIONAL: .5	OTHER: 1.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are to identify measures of learning and memory which change with age, to specify psychological processes and their relationships with age, and to develop procedures for improving learning and memory performance in the elderly.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Primary objectives are: (1) to identify which aspects of learning and memory change with age (and which do not); (2) to specify psychological processes underlying such age changes; (3) to identify health, nutrition, biochemical, and personality variables which are correlated with performance or with change in performance; and (4) to develop procedures to improve learning and memory performance in the elderly.

Methods Employed: Experiment XXXIII is a recently initiated longitudinal study which includes immediate free recall, delayed memory, dichotic listening, forward digit span, simple reaction time, and four levels of choice reaction time. These procedures (somewhat modified) were included in Experiment XXXI of this project. In immediate free recall, twelve familiar words are presented visually at a rate of one per second, and the task is to recall as many words as possible (in any order and without time limits) at the end of the presentation. Two delayed memory procedures are included. In delayed recall, the task is to recall as many words of the free-recall list as possible after an interpolated task (digit span). In delayed recognition, the twelve words in a free-recall list mixed with twelve other familiar words are presented in random order after the interpolated task; the recognition task is to identify which words are from the list and which are not. In dichotic listening, two different digits are presented simultaneously, one to each ear; the task is to identify the two stimuli. In forward digit span, lists of three to nine digits are presented at a rate of one digit per second, and the task is to respond with all the digits in a list in the order presented. In simple reaction time, a zero appears on a screen 15 times during a 90 second period, and the task is to press a button as fast as possible for each stimulus. In the four choice-reaction procedures, 90 digits appear at a rate of one per second, and 15 of these digits are target stimuli which are signals to press the button as fast as possible. The four rules for responding are: (1) a specific digit; (2) any odd (or even) digit; (3) any even digit which immediately follows an odd digit (or vice versa); and (4) any even digit which immediately follows an even digit, and any odd digit which immediately follows an odd digit.

Experiment XXXVI is an age study of memory for sentences which are logically related (e.g., "Jane is taller than Beth," "Beth is taller than Ann," "Jane is taller than Ann"). The study is designed to determine whether memory performance of educationally active young and old adults is affected differentially by linguistic integration based on inferences. Two acquisition conditions, one devised to provide high linguistic integration and the other to provide low linguistic integration, are included. There are six parts to the experimental procedure: (1) acquisition; (2) recall of names; (3) ordering of names: (4) recognition of sentences; (5) recall of information; and (6) inference.

(1) During acquisition, ten different sentences (of which five are repeated twice) are presented on a screen, one at a time. The sentences involve six names and their relative heights; each sentence compares two names (e.g., "Jane is taller than Beth").
(2) Recall of names determines how many of the six names are recalled.
(3) Ordering of names determines how well the ordering has been mastered (the names are provided during this task).
(4) In recognition, the ten different acquisition sentences are intermixed with five consistent sentences and six contradictory sentences, and the task is to decide for each sentence whether it had been seen during acquisition or not. A confidence rating is made for each judgment.

(5) In recall, 15 sentences in the form "Jane is shorter than Beth" are presented. About half are consistent with the acquisition information and about half are contradictory. The task is to decide for each sentence whether it is true or false. A confidence rating is made for each judgment.
(6) The inference task involves another set of 15 sentences in the form "Jane is shorter than Beth." The task is to decide whether each sentence is true or false; but unlike the recall task (which requires remembering the acquisition information), during inference the basic information necessary for ordering is provided.

Experiment XXXVII is a study of functional memory and aging. Participants of the BLSA who are administered the problem solving tasks in another study (Project Number Z01 AG 00064-20 LBS, Experiment XII) respond to multiple-choice and short-answer questions about the events in the problem-solving session and about spatial layout, scheduling, and other routine aspects of their involvement at the Gerontology Research Center. In addition, perceived importance of the information and estimates of self-accuracy are rated. Comparable questions are also answered in a follow-up telephone inquiry at home a week after the initial session at GRC.

Major Findings:
A major hypothesis in the study of speed and accuracy of information processing (part of Experiment XXXIII) is that age differences increase with increasing cognitive demands. If this hypothesis is true, slopes of regression lines of response time on age should increase from the simplest to the most complex of the five tasks in this study. Earlier analyses of the data for the men provided only weak support for this hypothesis. It is likely that the increased number of missed signals for the older men (providing fewer response times for them which were probably biased in the low direction) masked some of the effects of cognitive demand on the functions between response time and age. To explore this possibility, slopes were calculated only for the ten shortest response times (of 15 possible); current data yielded the following slopes (in sec/yr) for the five tasks:

Task	Men	Women
Simple	.00089	.00103
Choice 1	.00124	.00121
Choice 2	.00108	.00140
Choice 3	.00202	.00410
Choice 4	.00219	.00309

It is reasonable to categorize these tasks into three classes: (1) simple--only the 15 target stimuli are presented (no discriminations are required -- all stimuli are targets); (2) choice -1 and choice -2 -- 90 stimuli are presented (including 15 targets) and the cognitive demand is low (targets are rarely missed); and (3) choice -3 and choice -4 -- 90 stimuli are presented (including 15 targets) and the tasks are relational resulting in high cognitive demand (targets are missed). It is clear, for both men and women, that the slopes (increases in response time per year) are lowest for the simple task and highest for the complex tasks (choice -3 and choice -4), results quite consistent with the hypothesis.

Although age differences in recognition memory tend to be small, current data from Experiment XXXIII are showing modest age differences in delayed recognition of words for both men and women. A small sex difference in number of correct recognitions favoring the women is also emerging at each age level. This sex difference is accompanied by a small sex difference in response bias, the tendency to say "No, that word was not in the list." Using an index of response bias developed in this section (to avoid problems inherent in traditional indices of response bias when applied to age data), the men are showing a slight tendency to say "No" at all age levels, and this may be reflected in their lower number of correct recognitions compared with the women. The response bias index is not related to age for men or for women in these preliminary analyses.

Experiment XXXVI is a study of memory for logically related sentences. Data collection was completed for 32 young (18 - 23) and 32 old (60 - 69) students at the University of Maryland. The data have been scored and are being prepared for computer analysis.

Experiment XXXVII is a new study of functional memory. Questions have been prepared, and data collection has been initiated.

Significance to Bio-Medical Research and the Program of the Institute:
Memory and learning are central to experimental psychology, and some of the most striking and consistently reported behavioral age differences in the gerontological literature have been found in verbal learning performance. The experiments in this project are designed to identify basic mechanisms of learning and retention and to measure differences and changes in these functions that occur with age. In addition, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning and memory of the older person.

Proposed Course of Project: Six-and twelve-year repeat data will continue to be collected for Experiments I and II, longitudinal studies of serial and paired-associate learning. First-time data will continue to be collected for Experiment XXXIII, the longitudinal study of several cognitive tasks involving memory, attention, and response time. Data will be analyzed for Experiment XXXVI, the first study of memory for related sentences. Further studies of memory for sentences or text will depend upon availability of staff. Data collection will be completed for Experiment XXXVII, the new study of functional memory.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00066-20 LBS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Perceptual Retention and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Baltimore City Hospitals

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning and Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.3	.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are: (1) to investigate perceptual retention and interference; (2) to determine under what conditions age differences in retention are affected by interference; (3) to investigate processes of interference and perception; and (4) to describe age changes in nonverbal memory. The study in progress this year involves estimates of age changes in memory for designs.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: One general objective is to investigate the effects of interference in perceptual retention and in perception: (1) to determine whether aging results in increased susceptibility to interference; (2) to explore conditions which affect age differences in interference; and (3) to develop procedures for testing mechanisms which may account for the empirical findings. Another objective is to study non-verbal memory and the conditions which improve such memory, especially for the old.

Methods Employed: Experiment VII is a longitudinal study of memory for designs in which subjects attempt to reproduce visual designs from memory. The Benton Visual Retention Test is used, and the primary dependent measure is the total number of errors in all ten designs. Each design consists of geometric figures presented for ten seconds and then withdrawn. The task is to reproduce each design from memory. Subjects may take as much time as they need to draw the design. The current analyses include regression procedures to estimate age changes within birth cohorts and to estimate nonmaturational effects over time within age groups.

Major Findings: Experiment VII is the longitudinal study of memory for designs (Benton). Regression procedures recently developed in this Section have been applied to first-time measures to obtain estimates of age changes within birth cohorts and estimates of nonmaturational effects over time within age groups. When participants are grouped by date of birth and, for each birth cohort, performance is plotted on calendar time, the slope of the best-fit line is an estimate of age change for that cohort. Similarly when participants are grouped by age and, for each age group, performance is plotted on calendar time, the slope of the best-fit line is an estimate of nonmaturational effects for that age group. Previous regression analyses of 16-year data for the men indicated a clear increase in slopes (errors per year) with increasing age of birth cohorts, i.e., the earlier born, the larger the slopes (the larger the rate of decline in performance). These slopes are estimates of age changes and are consistent with the direct measures of change in the longitudinal analyses which showed the oldest men declined the most. However, when the 16-year data were analyzed in two 8-year periods for each cohort in an attempt to demonstrate replicability, the slopes were quite inconsistent. It was concluded that eight years was too short to determine stable slopes of performance on calendar time.

This year, 20 years of data were available (more than 1,000 first-time performance measures), and each cohort was divided into two 10-year periods (1960.8-1970.7 and 1970.8-1980.7). There were four birth cohorts with data in the 1960-1970 period which could be compared with sufficient data of their age peers in the 1970-1980 period. The means and slopes of the comparable (encircled) groups are listed in Table 1 (with sample sizes in parentheses).

Table 1

	Means		Slopes	
	1960-1970	1970-1980	1960-1970	1970-1980
Birth Cohorts				
1881-1890	7.3 (61)		.31	
1891-1900	5.6 (129)	7.9 (31)	.30	.72
1901-1910		6.0 (31)		-.24
1921-1930	3.0 (135)		.10	
1931-1940	3.2 (58)	2.8 (63)	.35	.03
1941-1950		2.5 (101)		.01

Although the comparable 10-year means are in reasonable agreement (given the small sample sizes of some of the groups), the comparable 10-year slopes differ markedly.

Similar analyses were made of the 20-year data when grouped by ages (rather than by date of birth) and separated into the two 10-year periods. Means and slopes for all seven age groups (both periods) are listed in Table 2 (with sample sizes in parentheses).

Table 2

	Means		Slopes	
Age Groups	1960-1970	1970-1980	1960-1970	1970-1980
80s	9.5 (17)	9.8 (10)	-.24	.57
70s	6.5 (99)	5.9 (41)	.14	-.09
60s	4.8 (129)	6.9 (15)	.03	-.14
50s	3.9 (170)	4.6 (23)	.14	-.13
40s	3.1 (169)	3.4 (32)	.11	-.01
30s	2.9 (109)	2.5 (105)	.14	.00
20s	2.3 (40)	2.4 (52)	.13	-.09

Again the comparable 10-year means are in reasonable agreement. (The mean of the 60s group in the 1970-1980 period seems high, but it is based on only 15 men.) The slopes for the age groups in each period are small (except for the 80-year-old groups which include few men) which is consistent with the previous 16-year age-group slopes (based on different age groupings). What is interesting about these slopes are the signs; the groups in the 1960-1970 period are consistently positive (again with the exception of the 80s), and the groups in the 1970-1980 period are negative or zero (except for the 80s). In the previous 16-year analyses of age-group slopes, the common slope for all age groups was .07 errors per year. Sampling could account for that; i.e., as the study progressed, the caliber of men who entered declined. The vocabulary measures were consistent with that interpretation; all age groups declined in vocabulary over the 16-year period. The current Benton age-group data suggest a reversal of that trend. In the early period, the caliber of new participants seemed to be declining, whereas in the later period, it seemed to be improving. Vocabulary measures for virtually the same subjects were analyzed to determine whether these data were consistent with that interpretation. The vocabulary slopes for all age groups were negative during the early period; i.e., scores decreased during that time. The slopes for the later period were not consistently in one direction; only the sixties and the seventies were positive (increasing), and the sixties group is quite small. The vocabulary data are consistent with the interpretation of a decline in the caliber of new participants during the early period but are not consistent with the interpretation of an increase in caliber during the later period.

At this point, there are two reasonable interpretations of the Benton birth-cohort data. If the 10-year slopes are acceptable estimates of age changes, then: (1) the substantial "change" for the men born in the 1880s and measured in the 1960s as they moved from their 70s to their 80s was found to be even larger for the men born in the 1890s and measured in the 1970s who also moved from their 70s to their 80s; but (2) the substantial "change" for the men who moved from their 60s to their 70s during the 1960s was not only not replicated for the men of the same ages during the 1970s, but was in the opposite direction. The other interpretation is that the intake of new men into the study during the 1970s was inadequate to provide meaningful 10-year birth-cohort slopes except possibly for the men born during the 1930s and 1940s. It should be noted that the intake was not planned to provide data for such birth-cohort analyses.

Significance to Bio-Medical Research and the Program of the Institute: The general idea that a person becomes more susceptible to interference as he grows older is well entrenched in gerontological thinking and is often used to "explain" age differences in performance. The evidence for this idea, however, is sparse and not consistent. One purpose of this project is to explore the generality of the age-interference hypothesis for nonverbal memory and perception. It is important, both for theoretical and applied reasons, to identify those conditions which are especially interfering for the old. Another purpose of this project is to describe age changes in nonverbal memory, to identify the correlates of those changes to improve our understanding of aging and this aspect of memory, and to study conditions which may improve such memory, especially for the elderly.

Proposed Course of Project: Data collection will continue for Experiment VII, the longitudinal study of memory for designs, for both men and women. The search for predictors and correlates of change in this performance will continue in efforts to understand why some older men decline whereas others do not.

Publication:

Arenberg, D. Estimates of age changes on the Benton Visual Retention Test. *Journal of Gerontology*, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00067-14 LBS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Learned Modification of Visceral Function in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 7.10	PROFESSIONAL: 5.15	OTHER: 1.95

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the interaction of behavior and physiology in man. One study investigates behavioral procedures (relaxation and biofeedback) in the control of blood pressure in patients with high blood pressure; a second study investigates heart rate biofeedback in patients with angina pectoris; another study investigates the physiological mechanisms of heart rate control during exercise in normal man; one study investigates bowel function in normal man and patients with irritable bowel syndrome; another study is designed to learn whether patients with histories of meningomyelocele and/or urinary and fecal incontinence can be trained to become continent; one study investigates age differences in autonomic response patterns; one study investigates age differences in electrodermal activity; one study investigates the behavioral and psychological characteristics of patients with angina pectoris who have been scheduled for coronary angiography.

Project Description:

Objectives: A. To evaluate the clinical effectiveness of relaxation and biofeedback in the control of blood pressure (BP) in patients with borderline or mild hypertension.

B. To determine if patients with angina pectoris can learn to slow heart rate (HR) and can transfer this skill to an exercise stress test.

C. To identify the mechanisms underlying learned, voluntary control of HR during exercise in normal man.

D. To determine whether patients with histories of meningomyelocele and fecal incontinence can become continent.

E. To develop and test behavioral training procedures for treating urinary and fecal incontinence in geriatric patients.

F. To describe age differences in individual patterns of autonomic nervous system response, and to determine if, with increasing age there is a decline in autonomic nervous system adaptability.

G. To determine the factors influencing the expression of the positive skin potential response--an electrical response often associated with the wetting and drying of the epidermis--in order to interpret the significance of age differences in positive skin potential response magnitude and frequency. Older people show a decrease in the frequency and magnitude of the positive skin potential response as compared to younger people.

H.. To determine the relationship between the prevalence of self-recorded episodes of angina pectoris to the prevalence of anginal episodes inferred by a physician; to determine the relationship of mood state or personality trait to various characteristics of angina pectoris; to determine whether behavioral or psychological measures of angina pectoris increase the specificity of the diagnosis of patients that can benefit from coronary artery bypass surgery thereby reducing the need for coronary angiography as a diagnostic technique.

I. To compare patients with irritable bowel syndrome (IBS) to those with lactose intolerance in order to determine whether there are unique psychological or physiological characteristics associated with IBS.

Methods Employed: A. Patients were selected from the outpatient service of the Columbia Medical Plan. All had been diagnosed as suffering from high BP. Study patients were limited to those receiving no antihypertensive medication but having clinically-determined diastolic pressures (DBP) $\geq$ 90 mm Hg, or to those patients receiving diuretic therapy (only) for their hypertension regardless of their clinically-determined BP. One hundred fifty-eight patients participated in this multiphasic, controlled study. Phase 1 was one month long and served as a baseline phase. All patients recorded their blood pressures 9 times/day (3 times upon awakening; 3 times during the middle of the day; 3 times during the evening). In addition, patients had their pressures taken approximately once weekly by a health professional. In phase 2, patients were assigned to one of three behavioral treatment groups: self-administered relaxation, self-administered BP biofeedback, or control. Assignment to behavioral groups in phase 2 was such that the three groups were matched on baseline BP, and such that approximately equal numbers of medically treated and untreated patients were in each group. Phase 2 was 3 months long. Phase 3 was similar to Phase 2 in duration and in nature of the treatments: control patients continued as controls; relaxation patients were subdivided into two groups--group A continued to relax, group B was trained in the feedback condition; and feedback patients from Phase 2 were divided into two groups similarly to the relaxation patients. In phase 4, control patients received six months of therapy (relaxation followed by feedback); patients on diuretic medication had their diuretics withdrawn if their DBP $\leq$ 86 mm Hg, and they were followed as before; patients who received only one therapy (relaxation or feedback) were offered the other treatment; patients who received both therapies were monitored for an additional 6 months. All patients in phases 2-4 continued to record their BP daily and were asked to have their BP taken weekly by a health professional.

B. A group of ten male patients experiencing angina pectoris in association with medically verified coronary artery disease were studied. All patients were referred from the outpatient service of Baltimore City Hospitals. Each patient was given an exercise stress test to determine the level of HR, systolic blood pressure (SBP), and the product of HR and SBP, the rate-pressure product (RPP), at which he experiences pain. Then he was trained during 25 sessions to attenuate his HR while exercising on a bicycle ergometer. Following such training he again received an exercise stress test to determine whether the training in the laboratory improved his performance on the stress test. Half of the group were control patients and exercised on the bicycle without feedback training. They also received pre- and post-exercise stress tests. Finally, the control patients received training in HR control as did the experimental patients. In addition, follow-up procedures enabled the assessment of the carry-over effect of training on daily routines.

C. Two studies were conducted with healthy, well conditioned subjects to assess the ability to attenuate HR while exercising. In Study I ten college athletes were studied. After an initial warm-up and maximal exercise test, each subject was asked to exercise on a stationary bicycle set at a work load which elicited 60-70% of maximal HR. The five subjects in the experimental group were instructed to attenuate HR and received visual feedback while five control subjects merely exercised. Control subjects subsequently received the same training as the experimental group in four additional sessions. A warm-up and maximal stress test was repeated after each phase of the study and respiratory, metabolic and sympathetic nervous activity was monitored. In Study II, designed to evaluate the metabolic consequences of bidirectioned HR control, a group of 15 experienced bicyclists were studied. They were asked to pedal on a stationary bicycle 500 kgm/sec, 70 rpm) during five exercise sessions. The first session was identical for all subjects and required exercise only. During sessions two to five, five subjects were instructed to increase HR and received feedback, five were instructed to decrease HR and received feedback and five merely exercised. Heart rate, systolic blood pressure, respiration and oxygen consumption was monitored.

D. This project is a continuation of a project reported last year. An additional 19 patients aged 5-15 with fecal incontinence secondary to meningomyelocele have been entered into the study.

Diagnostic evaluation and biofeedback training were accomplished using a rectal tube as follows: The patient was placed in the right lateral position and a hollow cylinder 1.2 cm in diameter to which two doughnut-shaped balloons were tied was inserted into the anal canal so that one balloon was surrounded by the external anal sphincter and the other was surrounded by the internal sphincter. A third balloon 5 cm in length was inserted through the cylinder into the rectum and was used to distend the rectum. Momentary rectal distensions from 5 ml to 60 ml of air in increments of 5-10 ml were injected and immediately withdrawn in the rectal balloon.

In addition to measuring the pressure change in the external sphincter balloon caused by external anal sphincter contraction, the electromyographic activity of the external sphincter and the adjacent gluteal muscles were measured during training sessions. Parents completed daily records at home of number and type of incontinent episodes and number of appropriate toiletings.

Training consisted of 3 phases: Phase 1 was a diagnostic procedure which assessed the ability of the patient to contract the external anal sphincter prior to training, and the minimum volume of rectal distension which the patient could perceive. In Phase 2 the patient was taught to emit an appropriate contraction of the external anal sphincter in the absence of rectal distension by providing visual feedback of the amplitude of his response and verbal praise. In Phase 3 the patient was instructed to contract the external sphincter whenever he perceived distension of the rectum by balloon. Beginning with the lowest level of rectal distension which the subject could perceive, the volume of rectal distension was gradually increased as the subject acquired the ability to overcome the dilation of the lower anal canal by voluntarily contracting the external anal sphincter. In addition to verbal praise, marbles exchangeable for toys were given for appropriate responses to children under age 10.

Each training session was 45-60 min long and consisted of approximately 25 training trials. Training sessions were scheduled at two-week intervals, and training was continued until the patient became continent or ceased progress.

E. Patient flow will be described separately for fecal incontinence and urinary incontinence.

For patients with fecal incontinence a physical evaluation and history is done by a gastroenterologist to identify, for exclusion patients with inflamatory bowel disease, tumors, or fistulas. This examination includes laboratory studies for parasites or occult blood in the stool and a barium enema to evaluate diverticulosis. Physical examination is followed by a rectal motility study to evaluate the following: (1) strength of the external anal sphincter muscle, (2) ability to contract the external anal sphincter on command, (3) resting tone of the internal anal sphincter, and (4) threshold (minimum volume) of rectal distension which can be subjectively perceived by the patient. Rectal motility testing is done by inserting into the rectum a tube which has attached to it two balloons for independently detecting variations in tonus of the internal and external anal sphincters. A third balloon is used to transiently distend the rectum.

After completing the initial evaluation, the investigator explains to the patient and his family the type of records of incontinence which are to be kept at home, and the bowel training program which the patient is to follow. The bowel training program consists of having the patient sit on the toilet for 10-min immediately after breakfast each day, and giving an enema at the end of the 10-min period if no bowel movement occurs for two days. The patient is given another appointment for 4 weeks after initial testing, and is advised that a research assistant will arrange a visit at the patient's home. The research assistant may visit one or more times during this 4-week period in order to accomplish the following: (1) ensure that the family understands how to keep the records and does keep them, (2) ensure that the bowel habit training program is being carried out appropriately, and (3) administer the Hopkins mental status exam and the Beck depression inventory.

At the second appointment patients are classified into one of three groups with disposition as follows: (1) if no longer incontinent, patients are scheduled for a follow-up appointment in 6 months and encouraged to continue on the habit training program, (2) if still incontinent but judged too cognitively impaired or depressed to participate in biofeedback training, patients continue in the bowel training program but with the addition of explicit rewards (rewards to be selected in discussions with the family) for periods of time without accidents, (3) if patients are still incontinent at the second visit and are able to cooperate in biofeedback training, they are given such training. Biofeedback training is similar to the rectal motility study except that the patient receives visual feedback and verbal praise to help him learn to contract the external anal sphincter with adequate strength when he senses rectal distention. Between biweekly visits during biofeedback training patients are instructed to contract and relax the external anal sphincter 50 times each day to strengthen this muscle.

The active treatment phase lasts 8 weeks during which time patients in the second and third category above (behavior modification and biofeedback) come to the laboratory once every two weeks for evaluation and/or further training. Home records of incontinent episodes continue to be kept. At the end of the treatment phase patients are scheduled for follow-up at 6 and 12 months. At these times the rectal motility study will be repeated.

Patients with urinary incontinence (involuntary loss of urine during waking, occurring at least 2/week) are given a physical examination and a medical history is taken by a urologist to identify for exclusion patients with bladder infection, bladder outlet obstruction, detrussor hyporeflexia, and paradoxical (overflow) incontinence. Examination includes urinalysis, cystometry, cystoscopy if clinically indicated, and for males, a prostate examination. A urologist carries out a cystometrogram to determine the following: (1) threshold volume of bladder filling which can be detected, (2) threshold volume of filling which produces a sense of urgency, (3) ability to emit and to inhibit bladder contractions at the volume of bladder filling which causes a sense of urgency, (4) maximum volume at which the patient can inhibit bladder contractions, and (5) competency of the bladder neck and distal urethral sphincter mechanisms.

After completing these tests the investigator explains to the patient and his family the types of records of incontinence which are to be kept at home and the bladder training program which the patient is to follow. The bladder training program consists in having the patient go to the toilet and attempt urination every four hours. The patient is given another appointment for 4 weeks, and is advised that a research assistant will telephone to arrange a visit to the patient's home. The research assistant visits one or more times during the 4-week period to accomplish the following: (1) ensure that the family understands how to keep the records and does keep them, (2) ensure that the bladder habit training program is being carried out appropriately, and (3) administer the Hopkins mental status exam and the NIA depression inventory.

At the second laboratory visit patients are grouped into the following categories: (1) If no longer incontinent, patients are scheduled for a follow-up appointment in 6 months. (2) If still incontinent but judged too cognitively impaired or depressed to participate in biofeedback training, patients continue in the habit training program but with the addition of explicit rewards (rewards selected in discussions with the family) for four-hour periods of time without accidents. Additionally, the bladder training routine described by Foxx and Azrin (1973), which involves water loading and rehearsal of appropriate toileting behavior combined with verbal praise and rewards, is carried out in biweekly visits to the clinic. (3) If patients are still incontinent at the second visit and are able to cooperate in biofeedback training, they are given biofeedback training to inhibit bladder contractions during bladder filling and to contract the distal urethral sphincter. This is similar to the cystometrogram except that the patient is given visual feedback and verbal instructions during bladder filling. Between biweekly visits, patients are instructed to contract and relax the pelvic floor muscles several times each day to strengthen these muscles, and they are instructed to practice stopping the stream during every micturation.

The active treatment phase lasts 8 weeks followed by follow-up visits at 6 months and 12 months. Cystometrograms are repeated, and ability to inhibit micturation is retested at these follow-up visits. Home records of the frequency of incontinence are kept throughout the 8 weeks of active treatment.

F. Test of the hypothesis that the ANS of the aged is functioning differently than that of young adults necessitates age comparisons on the interrelationships among ANS response measures. Comparison of age groups on the correlations between ANS response measures is inadequate because the ANS of young adults does not show a uniform response, and correlations among ANS response measures across individuals often are non-significant. The pattern of intra-individual ANS responses thus needs to be investigated. The concept of individual specificity is a means by which to do this. Individual specificity refers to the tendency of an individual to emit the same hierarchy of responses to all stimuli. For example, hypertensives have been reported to have a greater tendency than normotensives to respond to all stimuli with a pressor response. Comparisons of age groups on individual specificity reflects age differences in the adequacy of ANS function because if an individual's psychophysiological response pattern is consistent across stimuli, his response pattern often will be inappropriate to the demands of the situation. Fifteen subjects in each decade from 20 to 80 years were tested twice with a two year test-retest interval. Each subject received the following stimuli which were presented in counterbalanced order for 30 seconds: (1) cold pressor, (2) mental arithmetic, (3) time estimation, (4) incongruous slide, and (5) exercise. These stimuli were selected because they are effective (i.e., elicit responses) and diverse, the appropriate stimuli by which to assess specificity. Physiological response measures were breathing rate, skin potential, digital blood flow, heart rate, and systolic and diastolic blood pressure. Intra-class correlations were computed to assess individual specificity.

G. It is clear that the state of the skin prior to the occurrence of a positive skin potential response is one factor influencing response magnitude and frequency. That is, changes in the resistance of the sweat ducts and changes in the resistance of the epidermis (both of which vary in most experiments from sweat duct filling, corneal hydration, and sweat reabsorption) influence the expression of the positive skin potential response. It is not clear whether these factors can account for all of the variation in positive skin potential response magnitude or whether the response, in addition, reflects changes in sympathetic nervous system activation.

Eliminating the dependency of the positive response on the state of the skin is not a simple problem because change in sweat duct resistance have a different effect on response magnitude than changes in epidermal resistance. To deal with this problem different stimulus spacing procedures were used to independently vary sweat duct and epidermal resistance. Sweat duct resistance was varied by interspacing relaxation periods between stimulus presentations to allow time for the corneum to dry between each electrodermal response. Epidermal resistance was varied by presenting stimuli at very short interstimulus intervals to keep the ducts constantly full and allow only the hydration of the corneum to change. The stimuli were mental arithmetic problems of varying levels of difficulty. Dependent measures were skin potential and skin resistance levels prior to each response, the positive skin potential response, and the skin resistance response associated with each positive skin potential response. Each subject's data was factor analyzed to determine the number of components reflected in the four electrodermal measures.

H. Seventy-five consecutive adult male and female patients have thus far been recruited for this study, which requires a total of 100 subjects. They had been scheduled for coronary angiography at the Johns Hopkins Hospital cardiovascular laboratory because of chest pain and were contacted by a team member shortly after their scheduling (usually 2 - 3 weeks prior to their catheterization). They were telephoned, the study explained to them, and their participation solicited. A package containing a consent form, copies of both the anginal questionnaires and mood sheets, and franked return envelopes were mailed to prospective participants. If a patient returned a consent form, he/she was enrolled in the study. Patients were asked to complete one form for each anginal attack they experienced. Patients were instructed to complete this form retrospectively, after they had treated their angina in their usual fashion (e.g., nitroglycerin, rest) and the episode had subsided. Furthermore, they were instructed to follow any other advice given by their physician even if it prevented the completion of the questionnaire at that time. The questionnaire is designed to delineate the location, intensity, and character of the individual's pain. This information will be compared to the information elicited by the patient's referring physician as well as by the house officer who does the initial work-up upon admission for angiography. The patient's referring physician was contacted by mail and asked to complete a questionnaire regarding the nature of his patient's angina. The house officer immediately responsible for the patient's care while in the hospital also filled out the questionnaire. Patients also were given copies of the Profile of Mood States and were asked to complete a form once a day usually around dinner time. Patients were asked to continue record keeping after discharge from the hospital for a period of two weeks. Copies of the Cornell Medical Index and the 30-item Guilford-Zimmerman Temperament Survey Emotional Stability Scale from the GZTS were mailed to Subjects approximately one week before they entered the hospital for catheterization. Data are being analyzed in the following ways: 1) a behavioral description of the angina will be made, noting ranges of similarities and differences between patients, especially with regard to patients with normal coronary angiograms, 2) emotional correlates of anginal episodes will be analyzed by examining variability on the Profile of Mood States, and scores on the Cornell Medical Index and Emotional Stability Scales, 3) frequency and character of episodes as evidenced by self-report data will be compared to referring physicians' and house officers' analyses, 4) analyses of anginal patterns post-angiography and pre-angiography will be made.

I. Irritable bowel syndrome (IBS) is defined by the presence of abdominal pain and altered bowel habits in the absence of any evidence of organic disease. It is a common disorder, occurring in 8-14% of the population. IBS is believed to be a psychosomatic disorder because 70-90% of patients have abnormal scores on psychological tests and because half of IBS patients report that their symptoms began during a period of stress. However, the psychosomatic hypothesis is disputed. The following study was designed to determine whether there are unique physiological or psychological characteristics which distinguish IBS patients from a group of patients who have similar symptoms but which are accounted for by an enzyme (lactase) deficiency.

A questionnaire was designed which is administered to all adult patients referred to the Division of Digestive Diseases of Baltimore City Hospital with symptoms indicative of IBS. This questionnaire includes items to determine whether the bowel symptoms are made worse by stress, whether symptoms interfere with work or social activities, or whether the patient is depressed. Other questions are concerned with associated gastrointestinal symptoms.

In addition these patients are given a colon motility test to determine whether abnormal colonic motility can account for the symptoms. This test involves inserting a rectal tube with a balloon into the rectosigmoid junction 15 cm from the anal verge and inflating this balloon in a stepwise fashion by adding 20 ml of air every 2 min up to a total volume of 200 ml. In patients with IBS, this distention elicits spastic colonic motility which is recorded as pressure changes in the distending balloon.

During the first year this test and questionnaire were administered to 11 IBS patients and 13 patients with lactose intolerance (as determined by subsequent laboratory tests).

Major Findings: A. Two hundred twenty-four patients were contacted. Of these, 58 refused to participate and 39 withdrew during the baseline phase. Thus, 127 patients completed Phase 1 (baseline), 96 completed Phase 2, and 90 completed all three study phases. Reasons given for discontinuing participation--e.g., "I'm too busy"--reflect the likelihood that the demands placed upon the participants were quite burdensome. Only 2.2% (2 of 92) of the medically treated patients expressed contentment with continued diuretic therapy, and 2.3% (5 of 224) either refused to participate or withdrew during the baseline because they found self-monitoring of BP to be frightening. Among those who refused to participate, more were female and/or advanced in age.

Major conclusions resulting from the analysis of baseline data are the following: 1) Extensive, self-monitoring of BP is feasible and practical; 2) SBP rises throughout the day but is highest in the afternoon; 3) DBP falls from morning to evening but is highest in the afternoon; 4) intra-daily range of SBP but not DBP is higher among women than among men; 5) both SBP and DBP fall during a one month period of self-monitoring and regular clinical monitoring; 6) standard deviations and ranges of self-determined BP are highly intercorrelated, however, changes in professionally measured BP are poorly correlated with these indices of BP lability; 7) SBP levels, rates of decline throughout the baseline period and lability indices are correlated with age, but comparable measures of DBP are not correlated with age. Some implications of these baseline data for the treatment of hypertension in the elderly are: that over-treatment due to overestimation of BP is less likely if home-recorded BP are obtained using a comprehensive protocol; that a differential taxonomy of hypertension based upon patterns during the day might reveal important age-related differences in the natural history of high BP.

Major findings resulting from the second phase of the study are the following:

1) Both SBP and DBP continued to fall through the sixteenth week of self- and clinical monitoring as indicated both by negative slopes and by BP changes which are significantly different from zero.

2) Analyses of variance on the SBP and DBP levels during baseline (Phase 1) and during the first 35 days (Phase 2A) and the last 35 days (Phase 2B) of Phase 2 revealed the following:

a) During Phases 1 and 2A medicated, non-medicated, and combined groups all showed significant time-of-day effects on SBP and DBP levels, but there were no differences among the Control, Relaxation, or Feedback groups and no group X time of day interactions.

b) Analysis of Phase 2B data gave results which were similar to those above except that significant differences in DBP levels were observed between behavioral treatment groups of non-medicated patients and in the combined group.

c) Feedback and relaxation groups combined were compared with the control patients. There were significant phase and time-of-day effects for both SBP and DBP; but significant behavioral treatment effects were present for DBP only.

3) All medication groupings showed significant time of day effects on lability. Analysis of variance on the changes in standard deviations of SBP and DBP from Phase 1 to Phase 2A, Phase 1 to Phase 2B, and Phase 2A to Phase 2B, shows that

both BP lability indices decreased with time in the study for all groups. This decrease in lability also is seen in the ranges and standard deviations of the ranges in Phases 1, 2A, and 2B.

4) Analysis of changes in BP as a result of feedback or relaxation practice revealed that both treatments were effective in lowering BP. Furthermore, since the number of times the behavioral treatments were practiced did not differ significantly between behavioral treatment groups nor from phase to phase, but the extent to which BP was lowered during practice improved with time; it is clear that these patients were continuing to practice their respective techniques and that their skills at lowering BP were improving with practice.

5) Both SBP and DBP measured professionally were observed to decline with time during the study.

Major findings upon completion of the entire protocol by 90 subjects:

1) Acute effects (i.e., changes observed immediately after the practice of a behavioral technique);

a) Both relaxation (R) and SBP biofeedback (F) lowered BP acutely.

b) Improvement in performance of R and F with practice showed that they are learned skills.

c) Acutely, R and F were equally effective for lowering SBP, but R lowered DBP more.

2) Long-term effects (i.e., over the 6 month course of the treatment protocol);

a) BP declined for at least 6 months with regular monitoring and patient-involved assessment of BP data.

b) The greatest lowering of BP by behavioral intervention occurred during periods when BP tended to be highest.

c) A combination of R and F, with F preceding R, was better than either used alone and slightly, but not significantly, better than R preceding F.

d) The long-term effects of F were slightly greater than those of R.

On these bases, a staged, incremental behavioral treatment for borderline hypertension is proposed as follows:

Step 1: Self-monitoring of BP in conjunction with regular professional monitoring and significant patient involvement in data assessment.

Step 2: Behavioral intervention with F added to above if necessary after 1 month of Step 1.

Step 3: Addition of R to treatment steps 1 and 2 if necessary after 3 months of Step 2.

B. All patients completed the study. Seven of the ten patients demonstrated HR control during the post-training stress test. In some patients the recovery from the stress test also was improved. No significant improvement in clinical status was noted during a three-week follow-up.

C. In Study I the feedback condition resulted in attenuation of HR. The average HR increase was 42.6 bpm (SD=10.0) in the experimental group and 54.5 bpm (9.2) in the control subjects. A significant reduction in HR also was present when the control subjects crossed over to the feedback condition (54.5 to 49.9 bpm). The analysis of concomitant respiratory and metabolic data suggests that HR attenuation resulted in improved efficiency (a decrease in ventilation and oxygen consumption) with no change in local muscle work (as evidenced by no change in serum lactic acid). No significant change in serum catecholamines was observed.

In Study II bidirectional voluntary HR control was demonstrated in experienced bicycle riders. The average HR increase in the speeding group was 43.00 bpm (18.60), as compared to 34.34 bpm (6.86) in the slowing group. The no instruction subjects increase was 38.19 (8.46). The HR difference between the two feedback groups was significant. It was accompanied by a significant difference in total body oxygen consumption (1.703 l/min and 1.574 l/min) and ventilation (41.87 l/min and 28.24 l/min). This difference between feedback groups was mainly due to slowing group decreases.

D. To date 20 children aged 5-16 have completed the protocol or have been terminated for lack of progress. Seven were continent at the end of biofeedback training, 6 improved as judged by greater than 50% reduction in the frequency of incontinence, and 7 received little or no benefit (less than 50% reduction in frequency of accidents). An additional 16 patients are in training at present.

Successful outcomes have been associated with the acquisition of two skills, both of which appear to be necessary for continence: (1) The patient must learn to self-initiate bowel movements and must evacuate at least every two days. (2) The patient must acquire an ability to contract the external anal sphincter in response to rectal distensions of 10-50 ml air by a balloon.

E. Eight patients aged 65-86 have been entered into the treatment protocol for fecal incontinence. Causes of incontinence were weak external anal sphincter in 5, depression in one, rectal prolapse in one, and loss of sensation for rectal distention (post-hemoroidectomy) in one. Of the 5 patients with weak sphincter, 3 were continent at the end of training, one showed an 80% reduction in frequency, and one had just begun training. The depressed patient dropped out after two training sessions with minimal improvement. The patient with rectal prolapse improved minimally and was referred for surgery. The patient with sensory loss improved by 70% on a habit training program; she was not given biofeedback training.

Eight patients aged 68-88 have been entered into the protocol for urinary incontinence. Three suffered from stress incontinence; 3 from neurogenic bladder; one from multiple, small CVAs; and the eight patient used her incontinence as a factitious illness to manipulate her family. Two patients with stress incontinence became continent and one showed an 80% reduction in frequency. Two of the patients with uninhibited bladder showed an improvement of 80% or greater, and one is still in training. The patient with double incontinence secondary to CVA did not improve, and the patient with factitious behavior was continent only when monitored closely and reminded to urinate every 2-3 hours.

F. Consistent with hypothesis there was a significant increase in individual specificity with increasing age both on the initial test and the retest. That is, older people tend to have a more consistent pattern of response across stimuli than younger people and this is a replicable phenomenon.

G. Positive skin potential response magnitude varies as a function of the state of the skin prior to the response and does not vary with sympathetic activation. Thus, age differences in this response measure must reflect age differences in the peripheral sudomotor system.

H. Seventy-five of the total 100 subjects have been recruited. Data from 73 subjects are currently being scored, coded, and readied for computer analyses. No findings are available at this time.

I. A comparison of the two groups on questionnaire items indicates that IBS patients are not more likely to report that their abdominal pain is made worse by stress (1/11 for IBS, 8/13 for lactose intolerance), nor that their bowel symptoms interefere with their social activities (6/11 for IBS, 12/13 for lactose intolerance) or work (5/11 for IBS, 11/13 for lactose intolerance). IBS patients were no more likely to report depression (3/11 for IBS, 8/13 for lactose intolerance). Contrary to expectations, all these differences (except depression) are statistically significant ($p<.01$) showing greater psychological contribution to the symptoms of patients with lactose intolerance, than in patients with IBS. The associated gastrointestinal symptoms of gas, belching, location of pain, etc. were comparable for the two groups. Colonic motility data have not been analyzed yet.

Proposed Course of Project: A. A manuscript describing the results of the 1 month baseline phase is currently in press and a second, reporting the results of the two 3 month treatment phases has been submitted for publication. A third, analyzing blood pressure lability is in preparation. In addition, analysis of the follow-up data is underway. This will include the results from Phases 4-6 for those patients who served as controls in Phases 2 and 3, the results from Phase 4 for those patients who received only one behavioral treatment during Phases 2 and 3, and the results from appropriate follow-up phases for those patients who discontinued diuretic medication after completing treatment with R and F. Furthermore, a continuation study is being planned in order to investigate these behavioral interventions as supplemental therapy for patients receiving drug therapy more potent than diuretics alone.

B. This project has been terminated.

C. Studies are now beginning to examine voluntary control during isometric as well as dynamic exercises.

D. An additional 20 patients will be studied. The outcome of biofeedback training and maintenance of treatment effects will be compared on such parameters as sensitivity for perceiving distention, sensitivity for perceiving the response of squeezing the sphincter, age, intelligence, and level of lesion.

E. The project will continue until approximately 20 patients have completed training for fecal incontinence and approximately 20 have completed training for urinary incontinence. We will then move the program to a nursing home and operate it on an inpatient basis until similar numbers of patients have been studied if sufficient resources are available.

F. A third test is projected at ten years from initial test.

G. Data from older subjects will be analyzed to determine the peripheral sudomotor system component responsible for age differences in positive skin potential response magnitude and frequency. Studies will be conducted on the functional significance of the positive skin potential response.

H. If the results show that there are psychological correlates of angina, it should be possible to improve medical management of these patients through the use of appropriate behavioral strategies. If behavioral or psychological procedures improve the diagnosis of those patients who will benefit from bypass surgery, it should be possible to incorporate these procedures into the diagnostic assessment of patients.

I. We will continue to accumulate cases until there are 25/group. A standard psychometric test will be added to the protocol for new patients.

Significance to Bio-Medical Research and Program for the Institute:

A. Hypertension research has been hindered by the lack of reliable measures of BP and of a method for adequately determining baseline BP. This program makes a significant contribution toward both of these goals by providing reliable measurements which can be used for a baseline. This is especially important in the evaluation of hypertension in the elderly, where a strong iatrogenic effect can lead to differences between office BP readings and home BP readings which can lead to faulty diagnosis or treatment. The behavioral treatment of hypertension is a current topic of great interest, and a well-done study will settle many questions. A behavioral treatment is especially relevant to the elderly because they respond less reliably to medication, and because systolic hypertension is apt to be aggravated by increases in cardiac output which may be especially amenable to behavioral treatment.

B. Angina pectoris is a serious disease whose likelihood increases with advancing age. Furthermore, it is a disease which greatly limits the quality of life. Any technique which can improve the ability of such patients to perform in their daily lives will have considerable value.

C. Cardiovascular adjustments to physical conditioning are attributable to processes mediated through the nervous system as well as mechanical adjustments which take place in the heart, blood vessels and in working muscles. Very little is known about the way in which the neurally-mediated effects occur, or about the way in which these effects are determined by behavioral mechanisms. This research is fundamental in understanding these processes.

D. Meningomyelocele affects 0.1% of births, and approximately 40% of patients with meningomyelocele have fecal incontinence. Therefore, there is a large population of patients for whom these procedures have relevance. Additionally, we should be able to apply these procedures to adults with incontinence secondary to other types of CNS injury, and some of the behavioral training procedures which we are developing for preschool-aged children may be applicable to the treatment of older patients with fecal incontinence.

E. Most incontinence occurs in young children and in old people. The prevalence of incontinence in residents of nursing homes is 10-25%, and the prevalence of urinary incontinence in nursing homes 13-48%. Incontinence is at best an obstacle to normal social interaction, and is often the deciding factor in whether to institutionalize an older person as opposed to caring for him at home.

F. Diseases of the autonomic nervous system (e.g. hypertension, coronary artery disease) predominate in old age. The life history of the individual is likely involved in the development of these disease processes. Autonomic specificity is one aspect of an individual's life history--the development of an individual's autonomic nervous system response to different situations. The older person's tendency to respond consistently to all stimuli (individual specificity) reflects a decline in autonomic nervous system adaptability. In studying a person's pattern of autonomic nervous system response over time it may be possible to determine the significance of this maladaptive response pattern. For example, a lifetime pattern of an overreactive pressor response may be involved in hypertension. The study of individual specificity enables these proposed relationships to be investigated.

G. Adequacy of function of the peripheral sudomotor system is crucial for older individuals. There is a reduction in sweat gland response which impairs the older person's ability to regulate temperature. The morphological changes in the epidermis present not only a cosmetic problem but also may be related to a loss in the skin's protective function. This research program is involved in developing adequate measures of sudomotor function so that age-related alterations and their significance can be investigated in man.

H. Angina pectoris is a serious, age-related illness which affects about 5 million Americans with about 150,000 to 200,000 new cases each year. Well in excess of 100,000 coronary artery by-pass operations were done last year. Many patients who are screened for surgery are found unsuitable after coronary artery angiography reveals no significant atherosclerosis. Since angiography is a procedure which has a relatively high risk, any means of identifying patients who should not be operated before they are subjected to angiography would be valuable. Furthermore, any technique which improves medical management of these patients is sorely needed since current methods are of limited value.

I. These preliminary findings suggest, contrary to the prevailing view of gastroenterologists, that psychological factors do not play a major role in precipitating the symptoms of IBS, and they tend to support the alternative hypothesis that IBS is associated with a biological predisposition to react to a variety of stimuli with excess colonic motility.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00068-19 LBS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Operant Performance, Memory and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
OTHERS:	Donald Ingram	Staff Fellow	LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning & Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to analyze complex maze learning of young and aged animals, and to determine techniques which act to improve learning ability. Another goal of this project is to determine age differences in operant performance for young and aged rats or mice, and to determine factors which may act to improve performance, and also improve retention of, the learned responses.

Research previously included in this project and still continuing is reported now in Project Z01 AG-00063-14 LBS.

PHS-6040
(Rev. 2-81)

Project Description:

Combined into Project Z01 AG-00063-14 LBS.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00069-16 LBS

PERIOD COVERED October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Exercise, General Activity Level and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
	Donald Ingram	Staff Fellow	LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of this project is to determine the effect of voluntary wheel exercise upon behavior for animals tested in lifespan developmental research, and in addition to consider the effect of voluntary wheel exercise upon longevity. Another goal of this research is to increase the period of vigorous activity during later stages in the lifespan of the rat or mouse.

Research previously included in this project and still continuing is reported now in Project Z01 AG-00063-14 LBS.

PHS-6040
(Rev. 2-81)

Project Description:

Combined into Project Z01 AG-00063-14 LBS.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00075-03 LBS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Stress, Coping, and Personality in Aging Men and Women

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul T. Costa, Jr.	Chief, Section on Stress and Coping	LBS	GRC	NIA
Other:	Robert R. McCrae	Senior Staff Fellow	LBS	GRC	NIA
	John deFigueiredo	Senior Staff Fellow	LBS	GRC	NIA (5/23/81 resigned)

COOPERATING UNITS (if any)

1. Clinical Physiology Branch
2. Department of Dermatology, Baltimore City Hospitals
3. Section on Learning and Problem Solving

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Stress and Coping

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.0	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the effects of stresses, coping processes, and enduring personality dispositions on psychological and health outcomes. One study investigates sex differences in stress, coping, and personality; a second examines the influence of daily moods and stresses on clinical conditions; a third measures personality through interviews and projective tests; a fourth utilizes existing longitudinal data to examine age related constancy or changes in personality and personal adjustment; the fifth examines health perceptions as a function of personality, age, and biomedical status; the sixth investigates individual differences in personality and cognitive performance over the adult life span.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:
The program of the Section on Stress & Coping, LBS, is devoted to an investigation of the stresses faced by aging adults, the methods and strategies used by them to cope, and the effectiveness of their coping efforts. Stresses are viewed as events or difficulties which the individual encounters. They lead to attempts to cope, and the success of these coping efforts is judged by various measures of adaptational outcomes. Personality and life situations are seen as determiners of all three of these categories of variables. Personality is defined as the organization of mechanisms for adaptation or coping, and the three domains of personality in our model - Neuroticism, Extraversion, and Openness to Experience - each has important implications for the process of coping. The development of life structures - careers, friendships, marriages - similarly represent global and vital forms of coping with inner and social demands. It is within this broad context of an aging personality in a complex social environment that the study of specific coping and adaptational mechanisms will be made.

Specific Project Objectives:
A. To determine the nature and extent of sex differences in life stress, personality, and subjective well-being.

B. To determine prospectively whether emotional states or stressful events influence clinical conditions such as psoriasis.

C. To assess adult personality through a variety of non-self report methods.

D. To describe maturational change or constancy in personality and well-being, and to examine the effects on subjective well-being of smoking behavior and motivations in the context of personality and life stress.

E. To identify the determinants of perceptions of health or medical complaints, and to examine concurrently and predictively the relation between chest pain and psychological distress.

F. To analyze maturational changes in cognitive performance in relation to other psychological and medical variables.

Methods Employed:
A. The chief source of data for studies in this project is the Augmented Baltimore Longitudinal Studies of Aging sample, which consists of 424 men and 129 women who are participants in the BLSA, together with 183 wives and 16 husbands who are not themselves BLSA participants but who have agreed to complete questionnaires at home. Data from the NEO Inventory, a personality measure which embodies the model of personality employed in this section, were gathered on 363 men (83%) and 269 women (86%). The NEO Inventory consists of a 144 item questionnaire which measures 18 traits, six in each of three major domains of personality: Neuroticism, Extraversion, and Openness to Experience. Subjects respond to each of the items on a five-point Likert scale from "strongly disagree" to "strongly agree".

B. While the underlying cause of psoriasis has not been found, a number of genetic, physical, and perhaps psychological factors appear to affect its development and course. However, the role of psychological factors has been more difficult to establish. Patients report psychological stress-related events to be related to the first appearance of psoriasis or to exacerbations and a high proportion of patients with psoriasis have been reported to be emotionally maladjusted --a conclusion rejected by some reviewers. Furthermore, efforts to establish a relationship between psychological factors and the course of psoriasis have been inconsistent and difficult to interpret as all the studies have been anecdotal and retrospective. The purposes of the present study were to examine prospectively and simultaneously psychological factors and cutaneous disease in patients with psoriasis, along with appropriate control groups, in order to determine if a "psoriatic personality" exists and to examine the relationship between psychological factors and flares of the skin eruption. The first set of analyses examined the influence of stress on psoriasis retrospectively, by assessing stressful life events during the year prior to the study, and relating these to the presence and severity of psoriasis. Relationships between personality traits and subjective well-being and subjects' psoriatic condition were also evaluated. Controls consisted of spouses (similar environment), sibs (similar genetics) and unrelated patients with dermatologic disorders. The second set of analyses examined stress, psychological moods and psoriasis prospectively across time. Specifically, the hypothesis that outbreaks of psoriasis are preceded by periods of high stress and/or disturbed psychological mood was examined. Also examined were the temporal relationships between reductions in stress and mood and psoriasis.

C. Subjects for this study are participants in the Baltimore Longitudinal Study of Aging (BLSA). In order to supplement and validate the self-report questionnaires used in other studies, a number of other methods of assessment are being employed. Stress and coping interviews are being administered to BLSA participants on a six-year cycle. The interviews, which are video-tape recorded for later rescoring, intensively explore life stresses associated with family, friends, careers, and leisure activities. Inquiries are also made concerning the participant's methods and strategies used to cope with stress. Data from the interview are quantified by the use of the California Q-Sort, which is completed by the interviewer and a second rater who has seen video-tapes of the interview. On another visit to the GRC, a subset of participants are asked to take a battery of three perceptual-projective tests, including the Holtzman Inkblot Test, a psychometrically improved form of the Rorschach test; the Embedded Figures Test, a measure of the cognitive style of field independence; and cards from the Thematic Apperception Test (TAT), and the Projective Assessment of Aging Method (PAAM), a projective test designed for use with older subjects. A number of reliable coding systems exist by which to quantify data from the TAT. Data from all these sources will be analyzed in conjuction with objective data in classifying processes of coping, adaptation, and successful aging.

D. (1) In recent years, the issue of constancy or change in personality through the adult portion of the lifespan has become a major focus of attention. The stability position has received support from longitudinal studies that this Section has been conducting which show that the relative standing of individuals on personality dimensions is fairly constant over time. By far the greatest number of studies conducted on personality and aging have employed self-report instruments as the primary or sole source of data. This strategy has both advantages and disadvantages. On the positive side objective self-report inventories have a number of properties which make them preferable to observer ratings or projective techniques. But self-report inventories are also prone to certain problems: the transparency of the items make it possible for individuals so motivated to present themselves favorably or unfavorably; the use of a standard format for answering questions (yes-no, a likert scale, a rating bar) makes it possible for consistencies in the style of responding to distort the scores obtained from the instrument, and lead to spuriously high consistency or correlations. Issues of social desirability, acquiesence, and extreme responding have been the source of much debate among personality psychologists, and no definitive resolution has been reached. There has, however, been relatively little research on age trends in response sets. Some writers have argued persuasively that there have been enormous changes in the amount and kinds of testing that individuals of different generations have been exposed to, and this may introduce unwanted sources of variance in tests. Clearly, before any conclusions are drawn about aging and personality based on the use of objective personality measures, some information on these issues would be useful. Analyses of data from the Baltimore Longitudinal Study of Aging were undertaken to clarify these issues. The GZTS was given to all subjects with standard instructions. Answer sheets provide three response options: yes, no and "?", but subjects were instructed to use the "?" option only if they were completely unable to select yes or no. Following the suggestion of Guilford (1949), scales containing more than three "?" responses were invalidated. This criterion has been used in all previously reported applications of the GZTS conducted by the BLSA. However, this exclusionary principle may distort results. In particular, if age produces caution in responding, a disproportionate number of older subjects may be excluded, perhaps especially the most cautious. Because of that possibility, a new approach was adopted in the analyses to be reported here. All the GZTS answer sheets were keypunched, so that responses to individual items could be analyzed. In the original scoring system, "?" responses were not scored, and thus tended to lower the score of the individual. In the present system, responses were assigned a value of -1, 0, or +1, with the "?" represented as a neutral rather than negative value. The handbook for the GZTS (Guilford et al, 1976) lists three scales which have been developed to estimate the influence of certain response sets. These are called the gross falsification (GF) scale, the subtle falsification (SF) scale, and the careless deviancy scale (CD). The first two are intended to screen individuals who may be attempting to present an unduly favorable impression; the last is a scale composed of relatively rare responses, and a high score is interpretable either as careless responding or deviancy in personality. In addition, it is possible to measure at least three other response sets on the GZTS. The number of blanks was summed across all scales to give an index, as was the number of "?" responses. Items in the GZTS are roughly balanced on most scales, so it is possible to sum up the number of yes responses and consider it an index, not of any substantive personality trait, but of the tendency to acquiese to items indiscriminately. For each of these variables, cross-sectional, longitudinal, cross-sequential and time-sequential analyses were performed. In the repeated-measures analysis, 348 men ranging in initial age from 32 to 74 and retested after 4 to 8 years (mean interval = 6.6 years) were the subjects. They were classified into seven age groups, each spanning a six year interval. In the time-sequential analyses, 328 men who were tested in the time period from 1958 to 1964 were compared with 278 men tested between 1965 and 1971. They were cross-classified by the same seven age categories used in the repeated measures design. In the cross-sequential analysis, 345 men tested between 1958 and 1964 were compared with 285 men tested between 1965 and 1971. In this design, however, they were cross-classified according to their dates of birth, using seven birth cohorts of six year intervals from 1896 to 1932.

D. (2) Smokers have been characterized as being high in psychopathology and psychic distress. It might be argued from the evidence that smoking is detrimental to one's well-being. However, smokers often claim that there are compensatory psychological benefits which accompany the use of cigarettes for sedation or pleasure. Many people turn to cigarettes when they are nervous or unhappy, and believe that smoking relieves their distress. There is scattered evidence that quitting smoking is associated with increased reports of depression, anxiety, and other unpleasant symptoms in many people. One explanation for this apparent contradiction is that neuroticism or poor emotional health may precede and lead to smoking. Smokers may be less adjusted to begin with, and even with the hypothetical psychological "benefits" of smoking, may still be lower in well-being than non-smokers. Similarly, people may take up, resume, or increase smoking when they are in periods of particular stress. The lower well-being of smokers may reflect the fact that they are, at any given point in time, under more stress than non-smokers. Finally, smokers may be disproportionately located in one segment of society - e.g., the young, or those of lower socio-economic status. Conceivably, these characteristics, rather than smoking per se, may account for the findings. In recent years, investigators have attempted to classify smokers on the basis of the reasons which they give for smoking. A number of motives or reasons have been repeatedly identified. The premise that smoking might be helpful could be meaningfully qualified by specifying the kinds of smokers for whom it might be most appropriate. Thus, many people claim that they smoke purely for pleasure and relaxation. We do not expect that these people would show any reduction in distress, but they might show some enhancement in the happiness they find in life. The present research examined smoking behavior and motivations in the context of personality structures, life stress and environmental circumstances to test the hypothesis that for certain types of individuals in certain circumstances smoking can maintain or enhance psychological well-being. This hypothesis was tested by comparing the measured well-being of smokers and non-smokers matched on certain personality and environmental variables; and, within the population of smokers, by comparing smokers who had more and less appropriate smoking motivations. The subjects in this study were taken from a pool of 1101 men, all volunteer participants in the Normative Aging Study (NAS), a longitudinal study of normal health and aging. The sample is 98% white and is characterized by geographic stability, physical and psychological health at time of entry into the study, and volunteer status. All but the lowest SES groups are well represented. The definition and distribution of the four smoking groups was as follows: Never smokers, individuals who consistently claimed never to have smoked tobacco in any form, 25% (N = 276); Former smokers, people who formerly smoked cigarettes, but presently smoked nothing at all, 35% (N = 390); Cigarette smokers, those who regularly smoked cigarettes, but did not smoke pipes or cigars, 31% (N = 341); and, Mixed smokers, people who did not fit in any of these categories, 9% (N = 94). Over the course of 18 months, subjects were mailed questionnaires consisting of a battery of tests to measure personality, life stress, smoking motivation (or functions of smoking for the individual), and psychological well-being. A series of analyses were undertaken to test the hypothesis that for some individuals, certain kinds of cigarette smoking may contribute to psychological well-being. Measures of life stress, age, and the three personality dimensions of neuroticism, extraversion, and openness to experience were collected. Smoking status, smoking motives, and psychological well-being were also measured.

E. (1) The association of medical complaints with psychological distress is a widely recognized phenomenon known through clinical experience and a few large scale studies. There is, however, no direct evidence that specific complaints about chest pains follow this same pattern. A first study examined the relation between self-reported chest pains and two measures of psychological distress in a national probability sample of over 6000 men and women. It was hypothesized that individuals who report various kinds of chest pain will be lower in general well-being and higher in depression than are individuals who report no chest pain. Data for this study were collected as part of the Health and Nutrition Examination Survey (HANES) conducted by the National Center for Health Statistics during a period from 1971 to 1975. Subjects were part of a national probability sample of non-institutionalized men and women aged 25 - 74, and data were collected from 100 locations in the coterminous United States. The General Well-Being scale (GWB) was administered to 6913 subjects from all 100 locations; the Center for Epidemiological Studies Depression scale (CES-D) was administered to 2814 subjects from the last 36 locations surveyed. Details on the sampling procedures used are available from NCHS. In general, however, the sample may be regarded as a representative of the non-institutionalized U. S. population for the given age range. The GWB is an 18-item measure of subjective well-being which contains subscales on freedom from health worry, energy level, satisfying or interesting life, cheerful vs. depressed mood, relaxed vs. tense or anxious, and emotional-behavioral control. The CES Depression scale is a 20-item self-report survey which covers psychic expressions of depression including hopelessness, sadness, loneliness and anxiety. Poor appetite, crying spells, and sleep disturbance are also measured. The GWB and CES depression scales show a correlation of $r = -.72$ in the present sample. Three self-report items on chest pain were asked of subjects: Have you ever had trouble with any pain or discomfort in your chest? Trouble with any pressure or heavy sensation in your chest? Severe pain across the front of your chest lasting for half an hour or more? Responses were recorded as yes or no. 17.3% of subjects reported discomfort; 13.7% reported pressure; and 7.6% reported severe pain. Three-way analyses of variance were conducted using 10 levels of age (five year intervals), sex, and response to the chest pain questions as the classifying variables; and GWB and Depression scores as the dependent variables. Because of the large sample size and the disproportion of some of the cells, a stringent probability level of .001 was required for significance.

E. (2) Although the previous study (E1) investigates the association of perceived chest pain with measures of psychological distress in a large and representative sample, interpretation of any finding is obscured by a number of factors. Of these, the causal ordering of any relation is most ambiguous. It could plausibly be argued that depression and lowered well-being are the results of coronary heart disease (CHD), or the attendant angina pectoris, or the knowledge of the individual that he or she probably has a life-threatening illness. Indeed, this is probably the most obvious interpretation. But there are reasons to suppose that cause and effect operate in the opposite direction in this case. Chronic longstanding distress or emotional instability may precede and contribute to the development of chest pain perceptions. If this is the case, then knowledge of personality dispositions might prove useful in the prediction and diagnosis of anginal complaints, and thus in the diagnosis of CHD. In order to examine these alternative interpretations of the data and avoid the known confounding influence of sex, we studied the medical records of male participants in the Baltimore Longitudinal Study of Aging. Personality and self-reported health data were collected prior to the presentation of any evidence of CHD. Individuals who then developed angina and/or electrocardiographic (EKG) signs of CHD were compared with others who remained free of CHD in an extensive follow-up period. This study thus combines a physician's judgment of angina with longitudinal data which allow a much stronger basis for the inference of causal relations between angina and neuroticism. This study attempts to answer two research questions. (1) Is neuroticism a risk factor for CHD - i.e., are there pre-existing differences in emotional stability or health perceptions between individuals who will and those who will not subsequently develop signs and symptoms of CHD? (2) Do existing differences in neuroticism and/or somatic complaints predict anginal symptom reporting among individuals who will be diagnosed as having CHD? Subjects in this study were 124 volunteers in the Baltimore Longitudinal Study of Aging who have received medical examinations and psychological tests during the period from 1958 to the present, ranging in age from 33 to 84 at entry into the study. On the basis of these medical records, subjects were classified into several groups: 1. The Asymptomatic group consisted of 27 men, free of EKG signs, angina symptoms, or history of MI at entry. during the course of the study, they showed definite signs of IHD, using resting or stressed EKG ST-segment depressions as criteria. However, these men never reported history of MI nor angina in a follow-up period of from 5 to 15 years, and remained free of these symptoms at their last visit to the BLSA. 2. The Angina-Only group consisted of 10 men, free from EKG signs, angina symptoms, or history of MI at entry. During the course of the study, they were diagnosed as having probable or definite angina. However, these men reported neither history of MI nor EKG signs of IHD or MI (ST-segment depression or QRS wave) in a follow-up period of from 5 to 11 years, and remained free of these signs at their last visit to the study. 3. The CHD-with-Angina group consisted of 49 men, free of EKG signs, anginal symptoms, or history of MI at entry into the study. In the course of the study, they were diagnosed as having probable or definite angina, and in addition, they showed either definite EKG signs of MI or IHD, or a history of MI. 4. The control group consisted of 29 men, selected from several hundred, free of EKG signs, anginal symptoms, and history of MI at entry, and at last visit to the BLSA, 10 to 20 years later. Although free of CHD, no attempt was made to screen these subjects for other illnesses, and they should not be regarded as a "healthy" control group. These subjects were age matched to the other three groups, but were otherwise randomly selected from the population of subjects free from CHD and with sufficient follow-up interval. 5. Finally, since so few Angina-Only subjects could be found in this sample, nine additional subjects were chosen who had anginal symptoms at entry into the study, but who showed neither EKG signs of CHD nor a history of MI during a follow-up period of from 5 to 25 years. All subjects were given the Guilford-Zimmerman Temperament Survey and the Cornell Medical Index at their first or second visit. In every case, administration of these tests preceded the first appearance of EKG signs or anginal symptoms by at least one year. Age was used as a covariate in all analyses reported. In order to test the hypothesis that emotional instability predisposes the individual to the development of CHD, analysis of variance (with age as a covariate) was conducted contrasting the clearest CHD group, the CHD-with-Angina subjects, with the Control subjects. It was specifically hypothesized that control subjects would be higher on the GZTS emotional stability scale. In addition, differences in the nine other GZTS scales were also examined. In order to test the hypothesis that personality differences would predict differences in CHD signs and symptoms, analysis of variance was conducted using CHD group (Asymptomatic, Angina-Only, and CHD-with-Angina) as the classifying factor. It was hypothesized that Asymptomatics would be highest and Angina-Only subjects lowest in emotional stability. It was also hypothesized that a tendency toward bodily concern, as measured by CMI sections and total physical complaints, would differentiate the groups. Specifically, Angina-Only subjects, who report chest pain in the absence of EKG signs of ischemia, were hypothesized to be highest in somatic complaints in the period before the development of angina. Finally, in order to replicate results with a larger number of Angina-Only subjects, analyses were repeated including subjects who reported angina on entry into the study among the Angina-Only group.

E. (3) A recent review of the relationship between hypertension and personality concluded that the most promising directions for research included (a) determining the kinds of cognitive and behavioral treatments which are most effective, and (b) determining which types of intervention are most effective for individuals characterized by different personality traits. A recently completed study using biofeedback and relaxation on a population of borderline hypertensives demonstrated that a combination of techniques is useful in producing sustained decreases in blood pressure. In another study, personality by treatment interactions during electromyographic recordings were examined in a small sample of college students. Qualls and Sheehan reported that individuals open to absorbing experiences may benefit more from relaxation, while closed individuals profit more from a biofeedback approach. The present study attempts to confirm the Qualls and Sheehan findings using blood pressure data from a completed study, and personality measures to be administered. The specific hypotheses to be tested are that treatment conditions that require withdrawal from the external environment (relaxation) will be most effective or beneficial for persons with high capacity for absorbed attention, whereas for subjects with low or limited in their capacity for absorbed attention (closed to experience), conditions (i.e., biofeedback) that place an external, attentional demand on subjects will be more effective. Subjects will be 90 men and women, members of the Columbia Medical Plan aged 28 to 70, who completed the biofeedback and blood pressure study described in Z01 AG 0067, and their spouses, if married. Three questionnaires have been mailed to subjects: the Tellegen openness to absorbing experience scale, used in the Qualls and Sheehan study; the NEO inventory, a 144 item personality scale which measures the related concept of openness to experience as well as neuroticism and extraversion; and the NEO rating inventory, which is filled out by the spouses of the subjects and provides an independent non-self-report assessment of personality. Questionnaires would then be mailed back.

Follow-up postcards and phone calls would be used to insure adequate response.

F. The extensive literature on aging and cognitive functioning has documented that declines in performance are to be expected in a number of areas, including speed of reaction and short-term memory. The present study uses the Army Alpha Test, a general intelligence test with both "fluid" and "crystallized" components, which has been given to BLSA subjects over a twenty-year period. The test is administered individually under standard timed conditions. After scores are determined, the test is returned to the subject and he is given an opportunity to finish the test at leisure. Two sets of scores are thus obtained; a timed and an untimed set. All subjects were given Form A of the Alpha on their first visit. Thereafter, subjects were treated differently according to age. On their fifth visit to the GRC, after an interval of from four to eight years, subjects under age 70 were given a parallel Form B of the Army Alpha, in order to avoid practice effects; subjects over 70 were given retests of Form A at each annual visit. Thus, two sets of longitudinal data are available for analyses: (1) Retest data after four-to-eight years on Form B for 350 men initially aged 17-65, and (2) data from six administrations of Form A spanning five to eleven years for 51 men initially aged 66-84. An extensive series of analyses is being carried out to examine changes in Army Alpha intelligence performance in relation to age, health, personality and other variables.

Major Findings:

A. Since the NEO Inventory was designed to operationalize a three domain model of personality, it was hypothesized that a factor analysis of the scales would yield three corresponding factors. Among men, this finding would replicate earlier work on the structure of personality. However, the NEO model had never been tested on women. Results of Varimax-rotated principal component factor analysis are presented below:

	Factor I Neuroticism		Factor II Extraversion		Factor III Openness	
	Men	Women	Men	Women	Men	Women
Neuroticism:						
Anxiety	.83	.78				
Hostility	.66	.75				
Depression	.82	.81				
Self-Consciousness	.75	.73		-.31		
Impulsiveness	.61	.67	.35			
Vulnerability	.69	.76	-.35			
Extraversion:						
Warmth		-.34	.53	.51		
Gregariousness			.68	.81		
Assertiveness	-.34	-.33	.68	.54		
Activity			.68	.47		
Excitement Seeking			.70	.57		
Positive Emotions			.67	.53		

Openness to Experience:

Fantasy	.35	.33			.61	.65
Aesthetics					.65	.71
Feelings			.39	.31	.59	.63
Actions				.44	.53	.43
Ideas					.68	.70
Values					.58	.63

Note: All loadings < .30 omitted.

These data provide clear support for the NEO personality model for both men and women, because the hypothesized model is found for both sexes. Most importantly, this suggests that the structure of personality is essentially the same for women as it is for men. However, additional analyses showed that there are consistent differences in the level of individual traits in men and women in this sample. Men are significantly (p<. 01) lower in total Neuroticism, and in each of the six facets of Neuroticism except Hostility. This is consistent with findings reported last year using the Eysenck Personality Inventory N Scale. Although there were no sex differences in total Extraversion or Openness scores, there were significant differences in specific facets of each of these domains. Thus, men were higher in Asssertiveness and Excitement Seeking, and in openness to Ideas; whereas women were higher in Warmth, Gregariousness, and Positive Emotions, and in openness to Aesthetics, Feelings, and Actions. There were no sex differences in Hostility, Activity, or openness to Fantasy or Values. These findings demonstrate the need for multi-faceted personality measures like the NEO in attempting to understand sex differences in personality.

B. The mean proportion of affected skin ("severity"), averaged across reports, was 10.6%. Hence this sample contained only a few severe psoriatics. Patients showed a wide variety of patterns across time, although most were stable. Only a few individuals experienced several major fluctuations (over 20% change) in their condition during the study, while many individuals changed very little over the course of time. In comparison with norms, analyses of the Life Change scores showed that the sample as a whole had not been under particularly great stress in the preceeding twelve months. Comparison of psoriatics and non-psoriatics in terms of life stresses and other variables hypothesized to influence psoriasis showed no significant differences. Thus, there appears to be no evidence of a "psoriatic" personality; nor is there any retrospective relationship to stress in the present data. Statistical tests do not support the hypothesized relationships between flares of psoriasis and stress and mood. Paired t-tests showed that mood disturbance before flares is not significantly different, on the average, from mood after flares or mood during control periods. Similarly, patients do not experience any more, or fewer, stresses in the before-flare periods than they do during post-flare or control periods. With respect to reductions in extent of psoriasis, several of the comparisons reached significance. Patients experienced significantly more pleasant events and unpleasant events before reductions than after reductions. Also, patients showed significantly more mood disturbance before reductions than during control periods. However, neither of these findings held up across both comparisons, pre vs. post and pre vs. control.

C. To date, 159 subjects (including 43 women) have been interviewed, and Q-sort ratings have been made by the interviewer for 134 of them. Thirty women and 66 men have been given the Embedded Figures Test, the TAT-PAAM, and the Holtzman Inkblot Test (HIT). Eighty of the HIT protocols have been completely scored for a set of 22 standard variables. In order to establish reliability of scoring the HITs, an expert in Holtzman technique was employed to score the 80 complete protocols, and interrater reliability coefficents were computed for a subsample of 29 subjects who were also scored by a member of this Section. Coefficients ranged from .68 to .98, and most were above .90, indicating excellent interrater agreement on these variables.

D. (1) Repeated measures analyses showed a significant ($p< .05$) effect on the repeated factor for number of question marks, which increased from 6.2 to 9.6; acquiesence, which decreased from 132.1 to 128.9; gross falsification, which increased from 11.8 to 12.1; and subtle falsification, which decreased from 21.2 to 20.8. Age group differences were seen for the number of question marks, which were highest in the 68 - 74 group and lowest in the 50 - 55 group. In addition, there were two interactions: men aged 38 - 43 at first testing showed a decrease instead of an increase in question marks; and men 55 - 61 showed an increase instead of decrease in acquiesence. These results are somewhat puzzling; certainly they do not show a monotonic change in any of the response sets with age. Most of the changes were extremely small in magnitude, and if we require a significance level of $p< .01$, only two effects are significant: the increase in question marks, and the decrease in acquiesence. Neither of these longitudinal changes was mirrored in cross-sectional differences, suggesting that the changes are due either to time of measurement effects (i.e., a cultural change during the testing period), or to "practice" (i.e., repeated exposure to the test). Examination of the cross- and time-sequential analyses, conducted on samples of over 600 men, was revealing. Analyses of number of blanks, number of question marks, acquiesence, gross falsification, subtle falsification, and careless deviancy show no significant ($p< .05$) effects for aging/cohort, aging/time, cohort/time, or cohort/aging; nor were there any significant interactions. These data suggest that the marginal cross-sectional differences and interactions in the repeated measures analyses are best regarded as unreplicable error, and that the longitudinal changes in acquiesence and use of question marks are attributable to "practice effects". The fact that the former decreased while the latter increased by three items is suggestive: perhaps subjects felt pressured on the first administration to avoid question marks, and agreed to a few items which they were uncertain of. Some years later, perhaps, as experienced subjects no longer as reticent to assert themselves, they used the question marks when they felt they needed to. In any case, these data imply that response sets are not ordered by age. The longitudinal researcher may want to consider the effects of repeated administration of the same instrument, but the particular effect seen here is small in its overall influence on scale scores, and probably unique to instruments like the GZTS which provide a question mark but fail to score it. We have shown that such extraneous sources of variance as acquiesence, extreme responding, and social desirability are not themselves age-related. None of the response sets is substantially related to any of the GZTS scales, and it is therefore not the case that stability in these response styles could account for stability in personality scale scores.

D. (2) Specific hypotheses of interest were: Does smoking influence psychological well-being? Do particular kinds of smoking motives result in coping with distress or enhancing positive rewards and satisfaction with life? In a first set of analyses we considered the first question by examining the effects of age and smoking status on the Bradburn well-being scales. These analyses were repeated using neuroticism as measured by the Eysenck N scale as a covariate, and were replicated on the four administrations of the Bradburn scales. Results indicated that there was no consistent effect for either age or smoking status on positive well-being. When similar analyses were conducted on Negative well-being, a consistent effect was seen with age. Individuals in the young and middle groups had reported more negative affect on three of the four occasions, and this effect was still seen when Neuroticism was controlled. There was also a small and inconsistent effect for smoker status: at Time 4, Never smokers had the least negative affect. It was notable that, when Neuroticism was not used as a covariate, Never smokers were always lowest and current smokers highest in negative well-being, and that this effect twice reached statistical significance. Thus, the association between smoking and lowered well-being appeared to depend on the mediating influence of neuroticism. Finally, the same analyses were conducted on Affect Balance. No effect was consistently seen, and no effect at all appeared for smoker status. In this analysis, as in the two preceeding ones there were no statistically significant interactions between the classifying factors. Our hypothesis, however, stipulated that smoking might be beneficial only for a subset of individuals, whose motivations were "appropriate". Analyses were first restricted to the half of the sample higher in neuroticism. Individuals who currently smoked in order to reduce negative affect were reported to have had significantly less Negative Well-Being, and in addition to have been higher on Affect Balance and Personal Security, and lower on Hopelessness. Quite different predictions were made with regard to the other smoking motives. Although smoking for Addictive, Habit, or Stimulation motives were also associated with neuroticism, they were not specific attempts to deal with distress. Thus, we predicted no benefit for those who smoked for these motives. In all these analyses, age, SES, and measures of the three personality domains were used as covariates, so whatever effect emerged thus could not have been attributed to differences in the groups on these variables. Results are easily summarized: while there were repeated significant effects for various of the covariates, there were no differences attributable to smoker status or to motive status. Within the population of men high in neuroticism, neither smoking status nor any particular kind of smoking appeared to influence well-being. Tests of other hypotheses using the same general design were also conducted. We hypothesized that smoking for pleasure would enhance well-being - among the more adjusted who could psychologically "afford" to seek pleasures, or extraverts, since they were more disposed to experience positive affect, and may have been more susceptible to the enjoyment of smoking. As the results made clear, all of these hypotheses were unsupported; once again, neither motives nor smoking status appeared to influence well-being.

E. (1) In all analyses, sex and symptom reporting were significant ($p < .001$) main effects, whereas age was never significant. Subjects who reported pain or discomfort in the chest were lower in general well-being ($F = 553.85$) and higher in CES Depression ($F = 90.24$) than those with no pain. Subjects with pressure or a heavy sensation in the chest were also lower in general well-being (F = 459.67) and higher in depression (F = 86.92). Finally, the smaller group of subjects who reported severe pain across the front of the chest, lasting for half an hour or more, showed an identical pattern, being lower on GWB (F = 239.95) and higher in Depression (F = 56.81). The magnitude of difference is notable, amounting to about two-thirds of a standard deviation on the GWB scale, and about one-half of a standard deviation on the Depression scale. In all analyses, women showed significantly lower general well-being and significantly higher depression scores, although the magnitude of the sex difference was not as large as the symptom differences. None of the two or three way interactions reached the .001 level of significance. The large sample size allowed a replication of analyses restricted to non-whites. In these analyses, subjects were collapsed across age, since in the first analysis age was significant neither as a main effect nor in interaction with other classifying variables. Results from these analyses exactly paralleled the full sample findings: non-white subjects who reported chest discomfort, pressure, or severe chest pain were lower in total GWB and on each of the GWB subscales, and higher in Depression. The same sex differences were also found, and again there were no interactions.

E. (2) Comparison of individuals who subsequently developed CHD with angina and individuals who remained free of both CHD signs and angina over the follow-up period from 10 to 20 years showed no significant differences in emotional stability (F = 0.39, n.s.). Both groups began with comparable levels of distress-proneness. F tests also indicated that there were no pre-existing differences in any of the other GZTS scales, measuring such traits as sociability, masculinity, activity, ascendancy, and friendliness-hostility. These data provide no support for the hypothesis that the temperaments measured by the GZTS play a causal or etiological role in the development of CHD. These personality traits do not seem to predict CHD pathology. In addition, total physical complaints as measured by the CMI also fail to differentiate CHD with angina subjects from control subjects (F = 0.04, n.s.). It thus seems unlikely that CMI sums represent early signals of the development of CHD. When comparisons are made among CHD groups, however, a definite pattern emerges. As the Table shows, Angina-Only subjects are lowest in Emotional Stability and Angina-Only subjects are also highest and Asymptomaic subjects lowest, in total number of reported physical complaints on the CMI (F = 5.05, p< .01). In addition, analyses on the nine other GZTS scales, and on the specific sections of the CMI show that asymptomatics are significantly more Restrained in personality, and less likely to complain of Cardiovascular or digestive problems. They also have

Mean GZTS Emotional Stability and CMI Total Physical Complaints Scores for Asymptomatic, Angina-Only, and CHD-with-Angina Groups prior to the Development of Signs or Symptoms

	Emotional Stability	Physical Complaints
Asymptomatic	22.35	10.69
Angina-Only	16.11	17.50
CHD-with-Angina	20.75	13.89

better health habits. In each of these cases, the Angina-Only subjects showed the opposite pattern, with the least restraint, the most cardiovascular and digestive complaints, and the poorest health habits. These analyses were repeated on a sample which included in the Angina-Only group eight individuals who entered the study with anginal complaints, but showed neither EKG signs nor history of MI during a follow-up period of from 5 to 11 years. As might be expected, angina-only subjects were significantly higher in cardiovascular complaints on the CMI -- a finding which might be attributed to the angina of some of the members of the group. However, these same subjects were also significantly higher on complaints in the digestive, respiratory, musculo-skeletal, and skin sections, as well as total physical complaints ($F = 6.43$, $p < .01$). However, in these analyses, asymptomatics showed only a non-significant tendency toward being more restrained ($F = 2.81$, $p < .066$). They also showed a tendency toward being more masculine than other subjects ($F = 2.90$, $p < .061$).

E. (3) Up to the present time 38 participants have been entered into the study. Data collection is ongoing and no results are available yet.

F. Previously reported results showed that there is no evidence in these data for any effect of hypertension (within the present BP ranges) on Army Alpha performance among men. No other results are yet available.

Significance to Biomedical Research and the Programs of the Institute:
A-C. Psychological stress has long been considered a possible contributor to a number of illnesses; and several interventions have been designed to reduce stress and thus promote health. However, many questions remain about the proposed link. These include the identification of specific illnesses which are stress-related; the length of time between stress and subsequent illness; and the nature and mechanisms by which psychological states influence physical health. In the past, research on these questions has been hampered by the use of unreliable measures and retrospective designs. The program of research of the Stress and Coping Section can make substantial contributions to this area by its use of prospective designs and well-validated measures on a large population with documented medical histories. Research on personality may contribute to an understanding of the mechanisms linking stress and illness; at a minimum, it is essential to an understanding of the measurement of stress and illness. Research on stability and change in personality, on the developmental antecedents of Openness to Experience, and on the determinants of well-being and successful aging contribute directly to the mission of this institute.

D. (1) These methodological investigations contribute to the quality of research on aging in the important field of personality and adaptation.

D. (2) This study provides no evidence in support of the contention that smoking can be a useful tool in coping with distress or enhancing pleasure in life, and suggests that the "benefits" that smokers believe they obtain are illusory, a result either of changing nicotine levels or simple rationalization. There is no basis for recommending smoking as an aid to psychological well-being; and in view of the repeated warnings of the Surgeon General, cigarette smoking must be regarded as a hazardous form of coping. By considering the psychological implications of health threatening behaviors, this research contributes to the mission of the Institute.

E. Using an unselected sample, prospective design, objective personality assessment, and extensive follow-up intervals, the present research demonstrates that personality affects the presentation and perhaps the experience of anginal symptoms, and may aid in diagnosis. Since CHD is one of the major health problems of the elderly, informatiom on the etiology and diagnosis is particularly relevant to research on aging and health.

F. Cognitive declines, including senility, constitute the single most pressing health problem of the aged. Risk factors for senility are not currently known. The present research project may result in predictors of cognitive decline, and so contribute to this important research endeavor.

Proposed Course of the Project:
A. Sex differences in stress, coping, and personality will remain a major concern in on-going and future research. In addition to routine analysis of all data by sex, plans are being made for the inclusion of questionnaires specifically designed to measure stresses of particular relevance to women. The influence of potentially stressful events will be assessed by comparing the well-being levels of groups who have experienced different events. The presence of adaptation level effects will be assessed by comparing the perceived stressfulness of daily events in different age, socio-economic status, and life events groups.

B. Currently there are no plans to continue this project.

C. Data collection will continue. In the coming year, an emphasis will be placed on obtaining second Q-sort ratings on the interviews already video-taped. As subjects return, two-year longitudinal data on the projective-perceptual tests are being collected. Typed transcripts of the TAT-PAAM protocols will continue to be made preparatory to scoring. Plans for the incorporation of computer-assisted testing devices are being made; this would allow both controlled administration and immediate scoring of tests.

D. The longitudinal analysis of change or stability requires the collection of data over a period of many years. A battery of tests has been selected which will be administered by mail to BLSA participants and their spouses on a cyclical basis. Most tests will be re-administered after six years, at which time longitudinal analyses will be performed. The use of a 10% holdout sample, which will receive these tests for the first time in six years, makes possible the use of cross-sequential and time-sequential designs to help in distinguishing generational and times-of-measurement effects from true maturational changes. In the intervening six years, it will be possible to analyze data from new measures in conjunction with existing data from the GZTS, Activities and Attitudes Questionnaire, and other instruments. While true longitudinal designs cannot be used with different instuments, long-term predictive relations can be sought, and certain kinds of quasi-experimental designs can be used. Specific plans include an analysis of the effects of retirement and widowhood on subjective well-being.

E. (3) The project will continue until data collection is complete.

F. Further analyses of the Army Alpha are planned, particularly longitudinal studies of change in cognitive performance over an age span from 17 to 97. Analyses planned include: comparison of initial scores of those who returned for retest with those of dropouts (from whatever cause); speed vs. power, or determination of which sub-tests are most heavily influenced by the time constraints of the regular administrations; also, use of untimed as well as timed test scores in repeated measures analyses will allow an interpretation of the extent to which declines in test performance with age are a function of speed only, or speed plus some more basic intellectual functions. Finally, future analyses will examine the relations of age and personality dimension, specifically neuroticism, extaversion, and openness to experience with cognitive ability as measured by the Alpha. In particular, one hypothesis to be tested is that subjects who are more open to experience continue to learn and thus maintain intellectual performance longer than subjects closed to experience.

Publications:

McCrae, R. R., Costa, P. T., Jr., & Arenberg, D. Constancy of adult personality structure in males: Longitudinal, cross-sectional, and times-of-measurement analyses. Journal of Gerontology, 1980, 35, 877-883.

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McCrae, R. R. & Costa, P. T. Jr. Aging, the life course and models of personality. In T. Field et al., (Eds.), Review of human development. New York: Wiley, in press.

Costa, P. T., Jr., & McCrae, R. R. Stress, smoking motives, and psychological well-being: The illusory benefits of smoking. Advances in behavior research and therapy, in press.

Costa, P. T., Jr., Fleg, J., McCrae, R. R. & Lakatta, E. Influence of personality on symptom reporting and cardiovascular disease. Experimental Aging Research, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00076-02 LBS

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Openness to Experience and Coping Styles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert R. McCrae	Senior Staff Fellow	LBS	GRC	NIA
Other:	Paul T. Costa, Jr.	Chief, Section on Stress and Coping	LBS	GRC	NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Stress and Coping

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.5	PROFESSIONAL: .5	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the personality dimension of openness to experience and its relation to coping styles. One study investigates alternative techniques for the measurement of openness, including self reports and spouse ratings; a second study explores the relations between openness to experience and styles of coping and defense; a third study is concerned with the developmental antecedents of open dispositions in adulthood.

GRC/LBS-128

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:
Openness to experience is a broad dimension of personality which comprises a number of traits (including dogmatism, rigidity, openness to feelings, and need for variety) previously studied in isolation. In some personality systems, openness is viewed as the antithesis of defensiveness, and defense, in turn, is often seen as the core of all coping processes. This project attempts to measure a broad range of traits in the domain of openness utilizing a variety of assessment techniques. Simultaneously, measures of coping will be employed so that the relation between openness and styles of coping can be determined. In order to investigate the antecedents of openness to experience, research on the developmental history of individuals characterized in adulthood as open or closed has been done.

Specific project objectives:
A. To determine the correspondence between assessments of openness to experience and other personality traits as reported by the subject and as rated by the subject's spouse.

B. To investigate coping mechanisms and their relationship to age and to the disposition of openness to experience.

C. To examine parent-child relations as antecedents of openness to experience in adulthood.

Methods Employed:
A. Most personality research has relied exclusively on self-reports as the basis for assessing personality, although a tradition of using personality ratings also exists. Until recently, there was little data confirming the assumption that the results of these two assessment methods would be similar; and in fact some critics had argued that very different results would be obtained, thus casting doubt on the validity of personality measures. The NEO Rating Inventory, a third-person version of the NEO Inventory, which measures traits in the domains of neuroticism and extraversion as well as openness to experience, was administed to the spouses of BLSA members who had agreed to participate in this study. Responses were received from 136 men and 135 women whose spouses had also completed the NEO Inventory as a self-report instrument. Multi-trait, multi-method analyses were performed on these data in order to determine the convergent and discriminant validity of self-reported personality dimensions as judged by spouse ratings.

B. Subjects from the Augmented BLSA Sample were administered questionnaires to measure coping behaviors and styles. 287 subjects completed a Coping Questionnaire, and 151 subjects completed a Coping Self-Interview. The Coping Questionnaire required subjects to indicate which of 118 ways of coping they had used in response to a recent life event selected by the investigator. The items were taken from the Lazarus "Ways of Coping" scale, together with fifty new items developed from a review of the literature. In addition, subjects indicated which of the ways of coping had been most helpful in solving the problem, and in making them feel better. The Coping Self-Interview required subjects to select three recent events -- a challenge, a threat, and a loss -- and, for each one, to indicate which of fifty ways of coping they had used; whether it was helpful in solving the problem; and whether it made them feel better.

C. Based on developmental theories of personality, it was hypothesized that individuals who had been raised by parents who emphasized both love and freedom for the child, or, as Rogers terms it, "unconditional positive regard", should be highest in openness to experience, and especially in openness to feelings. Conversely, those who perceived their parents as less loving and more rigid in their demands should be closed to experience. The Parent-Child Relations (PCR) Questionnaire of Siegelman and Roe, which measures loving vs. rejecting, casual vs. demanding, and attention dimensions of childrearing practices, was administered to the Augmented BLSA Sample. Data from 329 men and 237 women regarding their fathers, and 339 men and 256 women regarding their mothers were obtained. Scores on the three dimensions of the PCR were correlated with the individual's scores on the NEO inventory.

Major Findings:

A. Correlations between self-reported traits and spouse traits on the NEO Inventory in the full sample ranged from .30 to .58 for the eighteen individual scales, and from .51 to .58 for the overall domain scores. Specifically in the domain of openness, correlations for men and women respectively were: fantasy, .42, .33; aesthetics, .66, .47; feelings, .35, .19; actions, .38, .36; ideas, .51, .46; and values, .47, .50. Total openness as self-reported showed correlations of .60 for men and .46 for women with openness as rated by spouse. Total openness also showed discriminant validity in low correlations with Eysenck Personaltiy Inventory E and N scales. These findings support a number of conclusions. First, and most importantly, there is evidence that the self-reports of openness to experience and other dispositions are not merely artifacts of one medium of assessment. The self-assessments are corroborated by the independent opinions of husbands and wives, who through long and intimate contact are well-qualified to judge the personality of their spouses. Second, these data suggest that some aspects of personality, such as openness to fantasy and feelings, are more difficult to rate than others, such as aesthetics and values. This may be because the latter traits are more observable. Finally, it appears that there is higher agreement when wives rate their husbands on openness than when husbands rate their wives. This may be due to the influence of sex-roles on the reporting or perception of personality traits in this area.

B. Factor analysis of the 118 items in the Coping Questionnaire led to the identification of 28 coping mechanisms. Taking one step at a time, restraint, rational action, expression of feelings, and positive thinking were the most frequently used mechanisms, whereas self-blame, intellectual denial, passivity, sedation, and hostile reaction were the least frequently used in the sample. Nearly half of the mechanisms showed age differences in use in response to a recent life event. However, the kinds of events which old people typically faced were systematically different from the kinds which young people faced; and these differences in type of stress were themselves strongly related to the choice of coping strategy. When statistical controls for type of event were used, six mechanisms showed age differences. In order to replicate these findings, analyses of the Coping Self-Interview were conducted, in which age and type of event were designed to be independent. Three of the six age differences found on the Coping Questionnaire were replicated using the Coping Self-Interview: Older (50-64 and 65-89) people were less likely to use hostile reaction, wishful thinking, or escapist fantasy than were younger (21-49) people. These cross-sectional differences tend to support the work of Vaillant and to contradict the notion that older individuals are prone to the use of primitive and immature defenses. In addition, the many age differences which were related to the type of event testify to the fact that older individuals are able to adapt their coping behavior to the changing situational demands of their portion of the lifespan. Older men and women do not rigidly maintain habits of coping which were appropriate in youth but which have outlived their usefulness. Instead, as stresses change, so do coping responses. This finding was replicated in the second study, where non-significant age-by-type-of-event interactions indicated that older people showed the same differentiated and flexible responsiveness to different types of stress that younger people did.

Several coping mechanisms were found to be associated with personality traits, including openness. Specifically, analyses of the Coping Questionnaire showed that open individuals tended to use substitution and humor in dealing with stresses, whereas closed individuals employed denial of affect and faith. These influences of openness on the choice of coping mechanisms were replicated in the Coping Self-Interview.

C. As hypothesized, closed men and women tended to perceive their parents as having been both rejecting and rigidly demanding in their childrearing practices. These conclusions, based on total openness scores, are seen most clearly in the facets of ideas and values. Contrary to hypotheses, openness to feelings was not significantly related to parental practices, except in the case of men open to feelings, who reported more love from their mothers. Although supportive of the hypotheses in general, the small magnitude of the observed correlations, ranging from .08 to .19 for total openness, demonstrate that these aspects of parental behaviors may not be as central in the development of personality dispositions as theorists have supposed. Many other influences, including peers, teachers, and other role models, may contribute significantly to the development of the disposition of openness.

Among men, extraverts reported demanding fathers and mothers, although there were no influences on extraversion among women. For both sexes, neuroticism was associated with rejecting parents, and these correlations, ranging from -.27 to -.33, were noticeably larger than the associations between parental relations and openness.

Significance to Biomedical Research and the Programs of the Institute: Successful adaptation to aging requires the constant use of coping. Medical researchers have been increasingly attentive to the role of individuals coping efforts in recovery from illness and management of chronic disease. However, no clear framework has emerged for ordering or classifying coping strategies, nor are there theories which would allow the specification of the most appropriate coping mechanisms for a particular individual or situation. This program of research is designed to examine a wide variety of coping and defensive processes in the context of a clearly defined and measured dimension of personality. If general principles relating personality dispositions to coping styles can be discerned, the application to specific medical problems can be greatly enhanced. In addition, the study of openness to experience in aging men and women contributes directly to the mission of this institute.

Proposed Course of the Project:

A. Additional analyses of the spouse ratings will be made in an attempt to determine variables which may moderate the relationship between self-reports and spouse ratings of openness and other personality dimensions. One such analysis will employ the Marlowe-Crowne Social Desirability Scale, which has been interpreted as a measure of defensiveness, and may be related to accuracy of reporting on self-report instruments. Since they have now been validated against self-reports, in future analyses spouse ratings can be used as an alternative measure of personality free from possible biases of self-report instruments.

B. Future analyses of the coping information will included detailed analyses of the roles of the individual and the situation in determining the choice of coping mechanisms, as well as analyses of data collected on the effectiveness of different coping mechanisms for solving problems and reducing distress. Plans for longitudinal administration of coping questionnaires will begin in order to determine if the cross-sectional age differences observed represent maturational changes, and in order to assess the stability of coping styles over a period of years.

C. No further research on the developmental antecedents of openness to experience is planned at this time.

Publications:

McCrae, R. R., & Costa, P. T., Jr. openness to experience in Loevinger's sentence completion test: Dispositional contributions to developmental models of personality. Journal of Personality and Social Psychology, 1980, 39, 1179-1190.

NIA Annual Report
October 1, 1980 through September 30, 1981
Gerontology Research Center
Laboratory of Cellular and Molecular Biology

The Laboratory of Cellular and Molecular Biology brings together various research projects that are related through a common interest in genetic information transfer, the biological aging process, and molecular mechanisms of physiological phenomena. The Section on Inorganic Biochemistry is concerned with fundamental studies at the molecular level of biological macromolecules involved in genetic information transfer. Age changes are investigated in systems involving these molecules, and particular emphasis is placed on the beneficial as well as harmful effects of metal ions that interact with these molecules. The Section on Macromolecules is concerned with the interaction of cell surfaces and receptors with synthetic substances, particularly macromolecules. These studies are aimed at the elucidation of cellular processes and the design of better drugs to interfere with such processes, especially those involved in diseases of the elderly. The Section on Cellular Aging and Genetics has been concerned with cell replication and repair of DNA damage, but the activities of this section are on a small scale, in comparison with activities before the departure of the former section head. Plans to build a new section which would utilize the new recombinant-DNA technology for studies of biological aging had to be abandoned because of the loss of positions.

However, for this year at least, we have had with us a guest worker on sabbatical leave, who has undertaken to utilize these techniques to assess whether methylation of particular genes changes during aging. Since such methylation has been related to gene inactivation, and since small age changes in methylation have been reported for whole tissues, the regulation or regulation failure of specific genes is a reasonable hypothesis for a component of biological aging. Results to date are preliminary, but appear to confirm the hypothesis.

It was previously shown in our laboratory that metal ions induce profound conformational changes in the structure of DNA. A particularly exciting conformational change was recently demonstrated for the dCdG hexamer, when crystals of the latter isolated from solutions containing divalent ions were shown by crystallography to be left-handed helices, in contrast to the usual right-handed helical structures of DNA. Evidence for the left-handed structure, called Z-DNA, has also been obtained by comparative circular dichroism analysis for polymeric DNA structures containing only G (guanine) and C (cytosine) bases. There is considerable interest in the scientific community in the possibility that localized conformational changes in DNA are important in the regulation of genetically controlled processes. We have therefore studied the ability of DNA [poly(dGdC)] to serve as a template for RNA synthesis during the transition from the right-handed B form to the Z form. Contrary to expectation we found that rather little change in RNA synthesis activity accompanies the transition. Also unexpectedly, we discovered that the transition from B to Z is often followed by a further conformational transition, to what we presently call the X-form, and this transition does appear to be accompanied by a dramatic decrease in RNA synthesis.

What errors does RNA polymerase perform most readily? Various circumstances, e.g. the presence of Mn^{2+}, can lead to errors in RNA synthesis, which can be classified as the introduction of (1) the wrong bases and (2) the wrong sugars. We have investigated which of the two types of error is most likely to occur by comparing the incorporation of deoxynucleotide having the correct base with ribonucleotide having the incorrect base. Using poly d(A-T) as template and ATP as a constant substrate, dUTP and CTP incorporation were used as the bearers of wrong sugar and wrong base, respectively. dUTP is much more readily incorporated than CTP, indicating that base fidelity is higher than sugar fidelity.

We continue to focus on the biochemistry of aluminum because of the elevated levels of Al found in Alzheimer's disease as well as in dialysis encephalopathy. Al inhibits RNA synthesis, but the inhibition is greatest when Al is preincubated with template, indicating that the major effect of the inhibition is due to the Al binding to DNA. NMR techniques have been used to confirm the structures of the Al-DNA complexes postulated on the basis of optical studies; namely, that Al^{3+} forms crosslinks between the bases of polynucleotide strands and that hydroxylated Al stabilizes the DNA through binding to phosphate. Two new peaks that increase with Al concentration have been identified in the ^1H-NMR spectrum of poly d(A-T) denatured in the presence of Al. One occurs upfield of thymidine H-6 and appears only in acidic solution (pH 5), where the crosslinking occurs and thymidine is therefore implicated in the crosslinking. The other occurs upfield of deoxyribose H-5', which is the phosphate-bound carbon; this peak occurs at acid and neutral pH, and thus confirms the existence of the phosphate-binding aluminum.

The interaction of Al with ATP has been studied in depth because of the postulate that ATP is the site of aluminum toxicity in dialysis dementia. Other laboratories have shown that Al inhibits brain hexokinase through ATP binding. A combination of ^{27}Al, ^{31}P and ^1H NMR has led to the identification of four different complexes, whose relative stabilities depend on pH. The pH-dependent dissociation of Al-ATP observed by NMR closely parallels the return of hexokinase activity (Womack and Colowick, PNAS 76, 5080 (1979).

Evidence has been obtained mainly from circular dichroism studies that different DNA conformations (e.g. B and Z) lead to different ways in which the DNA is packed together. The different conformations are observed as different low-intensity CD spectra and the differences in packing are observed as different high-intensity CD spectra [$\psi(-)$ if CD is negative, or $\psi(+)$ or $\psi^*(+)$ if positive). When B-DNA [as poly d(G-C)] is subjected to packing by polylysine, the $\psi^*(+)$ packing structure occurs, and when Z-DNA is similarly subjected to packing forces, a $\psi(-)$ type packing results. Thus metals can simultaneously determine changes in DNA tartiary and quarternary structures. A very clearcut demonstration of the relation between conformation and packing is the very different packing modes produced by treating the cis and trans $[Pt(NH_3)_2Cl_2]$ complexes of DNA with polylysine, producing packing. The cis-Pt is an antitumor agent, and it binds to adjacent G-bases on DNA. Trans-Pt forms crosslinks between DNA chains and between DNA and protein. The cis Pt-DNA complex is packed into a $\psi(+)$ form, and the trans Pt-DNA complex is packed into a $\psi(-)$ form. Thus the structural changes induced in DNA by the Pt determine the way in which the DNA is packed together.

Metal Ions and Chromatin Structure. We have previously studied age changes in the accessibility of DNA in chromatin. We are presently using metal ions as probes to study how the accessibility of DNA in chromatin can change. Chromatin is "melted" by heating and the absorbance monitored as a function of temperature. To obtain a melting profile the derivative of the absorbance is plotted vs. temperature by a computer program. The internucleosomal DNA melts at lower temperatures than the less accessible nucleosomal DNA. The presence of divalent ions decreases the temperature at which much of the DNA melts. The metal ion concentration required to produce a given decrease in melting temperature varies in the order $Mg^{2+} > Ca^{2+} > Co^{2+} > Zn^{2+} > Cu^{2+}$. This is the order of increasing tendency to bind to the DNA bases. The DNA that is less accessible and lower melting than nucleosomal DNA becomes clustered into several DNA fractions that are seen as peaks in the melting profile. The metal ions thus provide a technique for demonstrating the presence of DNAs of different accessibilities that cannot be detected in the absence of the metals.

Beta-Adrenergic Antagonists are extensively used to treat hypertension, cardiac arrhythmias, and angina pectoris. Numerous studies with these drugs show that the drug's effects are due to a blockade of receptors for adrenergic hormones, which are located on the surface of cells. A new derivative of a widely used drug of this class, alprenolol, was prepared. This derivative, $AlmCOCH_2Br$, has the potential not only to block, but also to destroy the receptor for adrenergic hormones. $AlmCOCH_2Br$ was extensively characterized *in vitro* systems and *in vivo* on rats of different age. Considerable differences were found between the results on senescent and maturing rats. In senescent rats up to 81-91% of the beta-adrenergic receptors in hearts and lungs could be blocked in an irreversible way. These results were similar to those obtained in maturing animals. In senescent animals the receptor number remains depressed even after one month, while in maturing animals the number returns to normal after 200 or 650 hours in hearts and lungs, respectively. The results point to the possibility of using an irreversible blockade to perform "molecular surgery" in senescent organisms, i.e. to excise part of the receptors in a practically permanent manner. Treatment by antihypertensive agents, a group of drugs to which beta-adrenergic blockers belong, is required for about 31% of the elderly. The above described results on beta-adrenergic blockers suggest that irreversibly acting drugs may gain a role in geriatric pharmacology. The development of drugs which would need to be administered only weekly would be of advantage since the total amount of drug used would decrease and senescent organisms are known not to handle xenobiotics well. Furthermore compliancy with the prescribed therapy would be easier to control. Although the approaches to the problems taken by the section are academic, they are by no means impractical. Support by the pharmaceutical industry, in the form of a stipend for one postdoctoral fellow, testifies to the practicality of the approaches taken; and the fact that this sponsorship does not have strings attached testifies to the fact that the integrity of basic research has not been compromised.

Lipid Soluble Vitamins, in addition to their obvious nutritional values, have further beneficial effects. Unfortunately to obtain a significant measure of these additional effects, doses which already elicit toxicity are required. Thus retinoids, which are derivatives of vitamin A, have been shown to reduce the incidence of lesions in epithelium induced by carcinogens. Work done in this program focuses on lessening the toxic effects of retinoids through transforming them to a water soluble form by complexation with solubilizing agents. Cyclodextrins, compounds obtainable in the processing of starch, were used as starting materials in the preparation of solubilizing agents. Substitution by alkyl groups in cyclodextrins yielded compounds which enabled the preparation of stable aqueous isotonic solutions that contained up to milligram amounts of retinoids per milliliter. The same solubilizing agents were found effective also in the preparation of stable aqueous solutions of vitamins D, E, and K.

Lowering the Ca content of the diet fed to young animals lowered the bone ash figure, and also delayed the onset of calcification. This rather surprising result indicates that this calcification mechanism is not only controlled by the bone inducing factor (which is supposed to convert mesenchyme cells to osteoblasts), but also by the Ca content of the milieu. Increasing the Ca content of the diet fed to old rats had no effect in accelerating bone formation. Nose studies on bone formation indicate that mesenchyme cells which may have the potential to become osteoblasts and form bone, and are plentiful in young animals, are very scarce or absent in old rats; and also that these animals lack a specific bone inducing mechanism.

Dipolar broadening of membrane protein spin labels has been used as a new probe of membrane mobility. ESR spectra of protein sulfhydryl spin labels show a mixture of two spectra. One corresponds to strongly immobilized labels and a second to weakly immobilized labels. By investigating the effect of temperature on erythrocyte and adipocyte membranes labelled at protein sulfhydryls, it has been possible to demonstrate the existence of a third class of sulfhydryl groups. It has been shown that this class of sulfhydryl spin labels generally have weakly immobilized signals which are partially broadened by the close proximity to other such sulfhydryl spin labels. At least on the erythrocyte, by using a sulfhydryl crosslinking reagent, it has been possible to show that these pairs of sulfhydryls are generally at the interface between protein molecules. The signal from these pairs of labels is very sensitive to the distance between them, which can be altered by temperature or other membrane modifications. This probe is thus sensitive to relative mobility of membrane protein molecules, unlike the other spin label and fluorescent probes which monitor fluidity in the lipid bilayer.

We have previously found that as a function of age the ratio of cholesterol to phospholipid increases. It was also found that this increase produces a decrease in the rate of lysis which is related to a decrease in membrane fluidity. In order to further investigate erythrocyte cholesterol, we have studied the effect of the oxidation of cholesterol to cholesten-3-one by cholesterol oxidase. The conversion of cholesterol to cholesten-3-one increases both the rate of lysis and membrane fluidity. These effects may be due to the elimination of a possible H-bond between the OH group of cholesterol and phospholipids. An important aspect of these studies is the dramatic difference between liposomes and cells. Thus in liposomes the cholesterol is all reacted within minutes, while under the same conditions the reaction with intact erythrocytes takes hours and has a very long lag. These differences must be related to the organization and accessibility of cholesterol in intact cells. It is expected that this may be a valuable probe for changes in membrane organization as a function of age.

Spin label studies have been undertaken in collaboration with the Clinical Physiology Branch in order to elucide alterations in these membranes with age. Using sulfhydryl spin labels we have found a dramatic irreversible increase in the weakly immobilized signal at 37°C. This effect is diminished in the presence of proteolytic inhibitors and has been shown to be due to the proteolytic partial disruption of the ghosts which liberate 10-15% of the protein into the ghost supernatant. These alterations make it impossible to perform aging experiments using these labels. However, we have begun a study of membrane lipid fluidity of 12 and 24 month old rat adipocyte ghosts using the stearic acid probes which dissolve into the phospholipid bilayer. These results suggest that the old ghosts are relatively more fluid at high temperatures but less fluid at low temperatures. This difference is due to a phase transition in the 10-15°C region which is more pronounced in the 24 month old ghosts than the 12 month old ghosts.

A study of iron in frozen hemoglobin solutions by Mossbauer and ESR techniques has been undertaken in collaboration with Johns Hopkins University in order to understand the perturbation of copper on hemoglobin which results in the oxidation of the iron.

We have found definitive evidence for a pair of new low spin iron species in the H_2O hydroxide, and to a lesser extent, the fluoride complexes of methemoglobin. The relative amount of this new species is greatly enhanced by slow gradual freezing of hemoglobin. A direct perturbation due to the freezing process is, however, ruled out by the finding that this species can be formed from the usual high spin and low spin species even at 210°K when water is already completely frozen. While these species were detected at all pHs between 9 and 5 the relative amount of this species increases below pH 7. This pH dependence is similar to that found for a conformational equilibria which exists in solutions previously shown to be associated with the coordination of copper. By comparing ESR spectra at 11°K of the imidazole complex it was shown that the new species have g-values close to, though not identical to, those of the imidazole complex. Complexes with similar, though not identical, spectroscopic parameters are also obtained at 87% glycerol where it has been suggested that the H_2O in the sixth coordination site is replaced by the distal histidine. Some similar equilibria between the distal imidazole as a sixth ligand and the weak water and fluoride ligands may explain the presence of these species.

Studies on brush border membranes have been undertaken in collaboration with the Laboratory of Molecular Aging with the ultimate goal of relating the transport phenomena studied in the Laboratory of Molecular Aging to alterations of membrane fluidity. Studies on the effect of cholecalciferol (Vitamin D_3) indicate that it decreases fluidity of liposomes but increases the fluidity of brush border membranes, while 7-dehydrocholesterol decreases the fluidity of membranes and liposomes. This dichotomy was explained by a cholecalciferol induced increase in phospholipid content of the membrane when it is incubated with liposome containing cholecalciferol. This decreases the ratio of steroid to phospholipid which is expected to increase fluidity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00044-08 LCMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Effects of Metals and Proteins on Nucleic Acids, Information Transfer, and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Inorganic Biochemistry

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 7.0	PROFESSIONAL: 5.2	OTHER: 1.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the interaction of molecules concerned with genetic information transfer. A primary objective is to determine under what conditions metal ions are essential for information transfer, and under what conditions they produce errors in the information and may thus contribute to biological aging. Topics of interest are: (1) the effects of metal ions on the structure of nucleic acids, nucleoproteins and chromatin; (2) age-related changes in chromatin structure; (3) aging of ribosomes; (4) the mechanism of involvement of aluminum in Alzheimer's disease; (5) crosslinking of nucleic acid strands by metal ions; (6) the effects of metal ions on RNA polymerase; (7) metal ions and cellular aging.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: (1) To study effects of metal ions on the structures involved in the replication, transcription, and translation of genetic information, (2) to determine how errors can be introduced into genetic information transfer by metal ions, and how these errors may affect aging, (3) to understand the interactions of proteins and nucleic acids and their mediation by metal ions, (4) to understand the role of metal ions in toxicity and aging, (5) to understand the participation of metal ions in the biological activities of nucleic acids and nucleoproteins, (6) to elucidate structural and functional changes in genetic information transfer that accompany aging.

Methods Employed: (1) The interaction of metal ions, proteins, and nucleic acids are studied by optical rotatory dispersion, circular dishroism, and uv spectrophotometry to determine conformational changes, and by infrared, nuclear magnetic resonance, and electron spin resonance techniques to determine interaction sites. (2) Chromatin structure is analyzed by means of model systems to determine which structural features are responsible for its function. (3) Physical chemical techniques are employed to study age changes in chromatin. (4) Effects of metal ions on nucleic acids and nucleoprotein are studied to determine under what conditions they serve an essential function in information transfer, and under what conditions they induce errors in information content. (5) The effect of metal ions on the enzymes responsible for genetic information transfer are studied. (6) The mechanisms by which enzymes and metal ions synthesize and degrade nucleic acids are elucidated.

Major Findings:

A. DNA Template Conformation and RNA Synthesis. It was previously shown in our laboratory that metal ions induce profound conformational changes in the structure of DNA. A particularly exciting conformational change was recently demonstrated at MIT for the dCdG hexamer, when crystals of the latter isolated from solutions containing divalent ions were shown by crystallography to be left-handed helices, in contrast to the usual right-handed helical structures of DNA. Evidence for the left-handed structure, called Z-DNA, has also been obtained by comparative circular dichroism analysis for polymeric DNA structures containing only G (guanine) and C (cytosine) bases. There is considerable interest in the scientific community in the possibility that localized conformational changes in DNA are important in the regulation of genetically controlled processes. We have therefore studied the ability of DNA [poly(dGdC)] to serve as a template for RNA synthesis during the transition from the right-handed B form to the Z form. Contrary to expectation we found that rather little change in RNA synthesis activity accompanies the transition. Also unexpectedly, we discovered that the transition from B to Z is often followed by a further conformational transition, to what we presently call the X-form, and this transition does appear to be accompanied by a dramatic decrease in RNA synthesis.

B. What Errors does RNA Polymerase Perform Most Readily? Various circumstances, e.g. the presence of Mn^{2+}, can lead to errors in RNA synthesis, which can be classified as the introduction of (1) the wrong bases and (2) the wrong sugars. We have investigated which of the two types of error is most likely to occur by comparing the incorporation of deoxynucleotide having the correct base with ribonucleotide having the incorrect base. Using poly d(A-T) as template and ATP as a constant substrate, dUTP and CTP incorporation were used as the bearers of wrong sugar and wrong base, respectively. dUTP is much more readily incorporated than CTP, indicating that base fidelity is higher than sugar fidelity. In fact, the incorporation of incorrect C base in CTP can be stimulated by the presence of U with either correct or incorrect sugar (UTP or dUTP).

C. Mechanism of RNA Polymerase Action. According to previous studies one of the major contributions of RNA polymerase to the proper functioning of RNA synthesis is to twist the incoming nucleoside triphosphate substrates in such a way that they will be in a proper position for hydrogen-bonding with complementary bases on the DNA template. Previously NMR techniques had been used to determine the distances between Mn^{2+} in the Mn^{2+}-activated enzyme and atoms in the substrate nucleotide, but no such studies have been available with DNA template present. When distances are compared in the presence or absence of template, these distances are very similar, and indicate that the template does not have much of an effect in orienting the substrate, thus confirming the effect of the enzyme in producing such orientation. It is assumed that the stability constant of the metal interaction is the same in the presence and absence of template.

NMR structural determinations of intermediates in enzymatic reactions are usually made on a stationary inactive system; in this case, the system is produced by using only one of two essential substrates, ATP, for RNA synthesis from a poly d(A-T) template. When UTP is also added to this system, RNA synthesis occurs, and we have followed the synthesis reaction in the NMR spectrometer, by monitoring the decrease in NTP β-phosphate and the formation of pyrophosphate. The reaction is accompanied by a decrease in the paramagnetic effect of Mn^{2+} on the atoms of the substrate, which we interpret as a destacking of ATP molecules by UTP molecules. Similar destacking occurs in the absence of the polymerase. Destacking may be an essential process in the initiation of RNA synthesis.

D. Biochemistry of Aluminum. We continue to focus on this metal ion because of the elevated levels of Al found in Alzheimer's disease as well as in dialysis encephalopathy.

1. Al and RNA Polymerase. Al inhibits RNA synthesis, but the inhibition is greatest when Al is preincubated with template, indicating that the major effect of the inhibition is due to the Al binding to DNA.

2. Al and DNA. NMR techniques are being used to confirm the structures of the Al-DNA complexes postulated on the basis of optical studies; namely, that Al^{3+} forms crosslinks between the bases of polynucleotide strands and that hydroxylated Al stabilizes the DNA through binding to phosphate. Two new peaks that increase with Al concentration have been identified in the ^{1}H-NMR spectrum of poly d(A-T) denatured in the presence of Al. One occurs upfield of thymidine H-6 and appears only in acidic solution (pH 5), where the crosslinking occurs, and thymidine is therefore implicated in the crosslinking. The other occurs upfield of deoxyribose H-5', which is the phosphate-bound carbon; this peak occurs at acid and neutral pH, and thus confirms the existence of the phosphate-binding aluminum.

3. Al and ATP. The interaction of Al with ATP has been studied in depth because of the postulate that ATP is the site of aluminum toxicity in dialysis dementia. Other laboratories have shown that Al inhibits brain hexokinase through ATP binding. A combination of ^{27}Al, ^{31}P, and ^{1}H NMR has led to the identification of four different complexes, whose relative stabilities depend on pH. At low

pH (1-2) we find a complex in which Al is bound to β and γ-phosphate. As the pH is increased (2-4), the β-phosphate bond is broken, and Al binds to γ-phosphate and N-7 of the adenosine base. Between pH 4 and 8, a third complex is formed by binding to β and γ-phosphate, and which contains stacked bases; the evidence shows that the Al is in three different environments in this complex, which seems to be a polymeric species. Between pH 8 and 11, a fourth complex is produced in which the Al binds only to γ-phosphate. The pH-dependent dissociation of Al-ATP observed by NMR closely parallels the return of hexokinase activity (Womack and Colowick, PNAS 76, 5080 (1979)).

4. Al and Other Phosphate Ligands. The interpretation of the NMR spectra of Al-DNA and Al-ATP complexes depends in part on comparisons of these spectra with those of a variety of other complexes of biological phosphate ligands. For this reason, as well as for their intrinsic interest, ^{27}Al and ^{31}P spectra were studied to characterize the binding of Al to triphosphate, ATP, dATP, GTP, pyrophosphate, ADP, CDP, GDP, GMP, AMP, and orthophosphate. All ^{27}Al signals are shifted upfield from $Al(H_2O)_6^{3+}$ for all these complexes, and they show splitting for bidentate ligands (e.g. NADP). The ^{31}P spectra are characteristic of the number of phosphate groups in the ligand and are not appreciably altered by other moieties in the molecules. Al broadens the signal for compounds with a simple phosphate. The Al-induced peak for diphosphates is both broadened and upfield shifted. Downfield-shifted peaks are observed only for triphosphates, where they occur along with upfield-shifted peaks. We conclude that upfield shifts are caused by direct binding of Al to a ligand, while downfield shifts arise from the binding of Al to other phosphates in the chain. Al binding to triphosphate resembles its binding to the phosphate component of ATP.

E. The Effects of Metal Ions on DNA Conformation and Packing.

1. NMR Studies on the Conversion of B to Z DNA. The common B form of DNA can be converted to the Z form by high concentrations of salt, and Z formation monitored by the appearance of a new peak characteristic of Z. We have shown that the conversion depends on the length of the DNA chains; high polymers convert more readily than small oligomers. Thus $d(pGpC)_6$ is only 20% converted to Z under conditions that lead to 100% Z form of poly d(G-C). d(A-T) hexamer and polymer do not undergo the conversion at all.

2. The Relationship between DNA Conformation and Packing. Evidence has been obtained mainly from circular dichroism studies that different DNA conformations (e.g. B and Z) lead to different ways in which the DNA is packed together. The different conformations are observed as different low-intensity CD spectra and the differences in packing are observed as different high-intensity CD spectra [ψ(-) if negative and ψ(+) or ψ*(+) if positive]. When B-DNA [as poly d(G-C)] is subjected to packing by polylysine, the ψ*(+) packing structure occurs, and when Z-DNA is similarly subjected to packing forces, a ψ(-) type packing results. Thus metals can simultaneously determine changes in DNA tartiary and quarternary structures.

A very clearcut demonstration of the relation between conformation and packing is the very different packing modes produced by treating the cis and trans $[Pt(NH_3)_2Cl_2]$ complexes of DNA with polylysine, producing packing. The cis-Pt is an antitumor agent, and it binds to adjacent G-bases on DNA. Trans-Pt forms crosslinks between DNA chains and between DNA and protein. The cis Pt-DNA complex is packed into a ψ(+) form, and the trans Pt-DNA complex is packed into a $\psi(-)$ form. Thus the structural changes induced in DNA by the Pt determine the way in which the DNA is packed together.

F. Metal Ions and Chromatin Structure. We have previously studied age changes in the accessibility of DNA in chromatin. We are presently using metal ions as probes to study how the accessibility of DNA in chromatin can change. Chromatin is "melted" by heating and the absorbance monitored as a function of temperature. To obtain a melting profile the derivative of the absorbance is plotted vs. temperature by a computer program. The internucleosomal DNA melts at lower temperatures than the less accessible nucleosomal DNA. The presence of divalent ions decreases the temperature at which much of the DNA melts. The metal ion concentration required to produce a given decrease in melting temperature varies in the order $Mg^{2+} > Ca^{2+} > Co^{2+} > Zn^{2+} > Cu^{2+}$. This is the order of increasing tendency to bind to the DNA bases. The DNA that is less accessible and lower melting than nucleosomal DNA becomes clustered into several DNA fractions that are seen as peaks in the melting profile. The metal ions thus provide a technique for demonstrating the presence of DNAs of different accessibilities that cannot be detected in the absence of the metals.

G. The Occurrence of Base Stacking in Metal-Nucleotide Complexes and Its Influence on Phosphomonoester Cleavage. Previous work with Zn, Pb and lanthanide-AMP complexes indicated that the formation of base-stacked networks, impeding reaction, underlay the differences in dephosphorylation activity of these complexes. Zn, in general, we argued, induced this kind of structure, lanthanides did not, with the properties of the Pb complexes falling in between. Thus, we hypothesized that the base stacking as induced by Zn adequately explained the inability of Zn to promote dephosphorylation. But there was not complete generality in the evidence, since base stacking - as indicated by CD enhancement - had been looked for and not found for Zn complexes of 5' nucleotides of G, C and U. Subsequently, we found such evidence for some Zn- and Pb-GMPs, but failed with other GMPs, as well as all CMPs and UMPs. Since the complexes tend to be insoluble, the polycation polylysine is added to solubilize them for CD study; however it is expected that the polylysine competes with the metal ions, and thus could break up the complexes perhaps to the extent that CD enhancement could not be observed. Recently we found that Pb-2'CMP was soluble (although aggregated) over a range of pH, and in the absence of polylysine gave CD enhancements suggesting possible modulation of dephosphorylation activity. The corresponding Zn-2'CMP was also soluble, but gave neither CD enhancement nor increased dephosphorylation. Therefore, it appears that organization into base-stacked networks can control dephosphorylation in Zn- and Pb-AMP complexes, and may in some others, but that it is not a general phenomenon of Zn-nucleotide complexes underlying the inability of Zn to promote dephosphorylation.

Significance to Biomedical Research and to the Program of the Institute: The participation of metal ions in every aspect of genetic information transfer and the deleterious effects on this transfer caused by undesired metal ions or essential metal ions in undesired concentrations make the study of metal ion interactions with nucleic acids of major importance. The possible relationship between aluminum accumulation and Alzheimer's disease and the discovery that the aluminum is bound to chromatin have emphasized the importance of studies on metal interaction with nucleic acids and chromatin.

An understanding of the structure and function of chromatin (and therefore protein - DNA interaction), ribosomes, the nucleic acid polymerases, etc. is essential to an understanding of cellular aging. We are particularly interested in studies that show how information transfer can go wrong. Metal ions are presumably not responsible for the primary events that cause aging but we believe that they may be important factors in determining individual and geographic differences in the aging process.

Proposed Course. We want to take advantage of the excitement in the biomedical community caused by the discovery of Z-DNA to demonstrate how the variety of DNA conformations including, but not limited to Z-DNA, can influence the genome. We shall study the biological effects of A, B, Z, ψ and other unlettered DNAs.

We shall continue our studies on the effects of metal ions on nucleic acid structure and function with emphasis on aluminum, because of its apparent relationship to Alzheimer's disease and/or to aging. We are expecting to receive some Alzheimer autopsy brain nuclei for comparison by NMR with chromatin that has been reacted with aluminum. We are encouraged by the studies on RNA polymerase and hope to further clarify the role of the DNA template in the mechanism of RNA synthesis, as well as to understand the differences in fidelity of the synthesis in the presence of various metal activators.

Publications:

Eichhorn, G.L., Butzow, J.J., Clark, P., von Hahn, H.P., Rao, G., Heim, J.M., Tarien, E., Crapper, D.R. and Karlik, S.J.: Metal Ion-Nucleic Interactions, Aging and Alzheimer's Disease. In Martell, A. (Ed.): Inorganic Chemistry in Biology and Medicine. American Chemical Society, Washington, D.C., 1980, pp. 75-88.

Eichhorn, G.L.: Bioinorganic Chemistry. In: Encylopedia of Science and Technology. 5th Edition. McGraw Hill, New York, in press.

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Karlik, S.J., Eichhorn, G.L., Lewis, P.N. and Crapper, D.R.: Interaction of Aluminum Species with DNA. Biochemistry 19: 5991-5998, 1980.

Eichhorn, G.L. and Marzilli, L.G. (Eds.): Advances in Inorganic Biochemistry, Volume 3, Metal Ions and Genetic Information Transfer, Elsevier/North Holland, in press.

Eichhorn, G.L.: The Effect of Metal Ions on the Structure and Function of Nucleic Acids. In: Eichhorn, G.L. and Marzilli, L.G. (Eds.): Advances in Inorganic Biochemistry, Volume 3. Elsevier/North Holland, in press.

Eichhorn, G.L., Tarien, E., Elgavish, G.A., Butzow, J.J. and Shin, Y.A.: The Effects of Metal Ions on DNA Proteins and DNA Polypeptide Complexes. In Sarma, R.M. (Ed.): Proceedings of the Second SUNYA Conversation in the Discipline Biomolecular Stereodynamics, Volume II, in press.

Butzow, J.J., McCool, M.G. and Eichhorn, G.L.: Does the Capacity of Ribosomes to Control Translation Fidelity Change With Age? Mechanisms of Ageing and Development 15: 203-216, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00112-01 LCMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

DNA Methylation in Aging Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Gunther L. Eichhorn	Chief, LCMB	LCMB NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Inorganic Biochemistry

INSTITUTE AND LOCATION

TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

DNA methylation of the mouse middle repetitive A particle genes and the mouse globin genes is being examined as a function of age. The method involves parallel digestions of mouse DNA with the restriction endonucleases Msp I and Hpa II, Southern blotting, and hybridization with nick translated probes. The present evidence indicates that some A particle sequences decrease in methylation during aging, while the entire genome in various tissues remains relatively constant in methylation between 6 and 31 months of age.

In addition, a new RIA for 5meC has been developed, based upon inhibition of binding between radioactive 5 methyl cytidine and anti-5 methyl cytidine. This RIA will be used to assess age-related changes in overall methylation of germ cell DNA.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To assess whether or not methylation of particular genes changes during aging, thus potentially affecting transcription of the genes. To establish a radio-immunoassay (RIA) for 5 methyl cytosine, the major eukaryotic DNA methylated base. To isolate anti-5mC via affinity chromatography and use it to further characterize age-related changes in methylation.

Methods Employed: DNAs are prepared via the standard Marmur extraction. They are restricted using various nucleases, particularly the pair Msp I and Hpa II, which have the specificities given below:

	-CCGG-	$-\text{C}^{m}\text{CGG}-$	$-{}^{m}\text{C}^{m}\text{CGG}-$*
Msp I	+	+	-
Hpa II	+	-	-

*Rare structure; over 90% of 5mC is followed by G.

The DNAs are then electrophoresed on agarose gels, stained with ethidium bromide and photographed, then denatured and blotted on nitrocellulose paper as described by Southern. The blots are hybridized with probes prepared via nick translation using α^{32}P-labeled nucleotides. After washing the blots, they are autoradiographed. In some cases, probes have to be isolated from pBR322 plasmids before being used. This part of the experiment is performed in R.C. Huang's laboratory under P_2-EK_2 containment.

The antibodies are prepared essentially as described by Levine *et al.* Affinity chromatography is used to purify anti5mC as described by Sager *et al.* The RIA for 5mC was patterned on that of Vold.

Major Findings:

A. General Methylation Status of Whole Genomic DNA of C57Bl/6J Mice. The evidence suggests that this parameter is relatively constant during aging. Genomic DNAs from liver, kidney, and bone marrow produce distinctly longer HpaII fragments than Msp I fragments at all ages studied (range of 6 to 31 months). Evidently most sequences of this type are in the form C^{m}CGG. In spleens of 7 and 31 month old mice, the Msp I and HpaII fragments are similar in length, suggesting that more-CCGG-sequences are found in this tissue.

B. Methylation of Specific Gene Sequences. Only genes of the A particle have been used as probes so far. Probe 1 is a sequence that is found in most copies of the moderately repetitive A particle family. I found this to be the case throughout the lifespan. Probe I hybridizes to DNAs that are very long in HpaII digests from all ages. The Msp I digests contain longer fragments recognizing probe I in younger animals. This preliminary result indicates that some sequences are present in the doubly methylated form (${}^{m}\text{C}^{m}\text{CGG}$) in young animals but become singly methylated (C^{m}CGG) in older animals, probably via DNA repair or replication with incomplete methylation. Such changes could release some of these virus-like genes for transcription.

Probe 2 is a sequence containing the A particle terminal redundancy. Preliminary results indicate that this sequence cannot be cut by EcoRI as effectively in old animals as in young animals. The methylation of this sequence is undetermined at present, but changes in EcoRI restriction in response to methylation changes have been described previously.

These results suggest that changes in methylation of the retrovirus-like A particle sequences may be age-dependent.

C. RIA for 5 Methyl Cytosine. A radioimmunoassay for 5 methyl cytosine having a high degree of specificity has been worked out. The anti-5mC is bound to tritiated 5 methyl cytidine in this RIA, and the unlabeled test compound is added to the reaction to inhibit the prepitation of radioactivity by means of binding to the antibody. Results show that unlabeled 5 me cytidine is the best competitor of radioactive 5 mC binding. ¢ X174(+) strand virion DNA, with 1 methyl group per 5.3 kilobase genome, is next most effective. ¢ X174 RF, the replicative form of the same genome, lacking methylation, is a very poor competitor, as are cytidine, poly C, and poly dGdC. Inhibition curves for these compounds are attached.

Over the last several years, a body of evidence has developed linking methylation of genic DNA to form 5 methyl cytosines, with inactivity of the methylated genes. Methylation is accomplished by methyl transferases, after replication. This correlation between methylation and shutting off of transcription is particularly striking for viruses, having been demonstrated for the following viruses: adenovirus type 2, adenovirus type 12, Herpes saimiri, and mouse mammary tumor virus. Other genes known to exhibit correlations between methylation and lack of transcription include β globins and fetal globins.

Some years ago, Vanyushin and collaborators analyzed the total 5 methyl cytosine content of DNA of male rats at 1, 12, and 28 months of age. This level decreased in a number of tissues. In brain, for example, it went from 1.10 ± 0.02 at 1 month to 0.94 ± 0.03 at 28 months. This quantitatively small change could have a significant impact on gene expression if most of the unmethylated sites in the older animals were localized near particular genes. The impact would be maximal if these genes included tumor virus genes integrated into the animal's own genome. It is generally observed that tumor incidence increases with age in a strain-specific way, implying a degree of genetic control over tumorigenesis. Regulation of DNA methylation near genes required for transformation might well provide such a control of this process. It is known, for example, that the concentration of S-adenosyl homocysteine, a potent methyltransferase inhibitor, does increase with age in a number of tissues. If this hypothesis holds, one could detect decreased methylation in various genes during aging, but the decreases in methylation ought to be particularly striking in genes of integrated viruses. A particle genes represent the first test of this idea, and they do appear to become less methylated during aging.

Proposed Course. The characterization of A particle probes 1 and 2 in aging DNAs will be completed. In addition, mouse globin genes will be examined similarly to see whether or not they are methylated differently in old animals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00046-11 LCMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Potential Drugs for Diseases Prominent in Senescence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	J. Kusiak	Special Expert	LCMB NIA
	J. Milecki	Visiting Fellow	LCMB NIA (EOD 12/3/80)
	L. Szente	Visiting Fellow	LCMB NIA (EOD 5/12/81)

COOPERATING UNITS (if any)
Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, Tennessee; Key Pharmaceuticals, Miami, Florida; University of Florida, Gainesville, Florida.

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Macromolecular Chemistry Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 4.2	PROFESSIONAL: 3.2	OTHER: 1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two groups of drugs which are heavily used by the elderly have been subjected to molecular manipulations in order to learn how their pharmacological profiles can be improved. In the group of beta-adrenergic blockers it has been established that introduction of chemically reactive moieties into drugs may have considerable therapeutic potential. The chemical reaction between such a drug and its receptor in tissue was found to make the effects of the drug longer lasting in maturing rats; in senescent rats the effects were prolonged dramatically and were noticeable even one month after the drug was applied. These findings point to the possibility of developing drugs which would have to be applied only a few times during senescence. In the field of fat soluble vitamins new potent solubilizing agents were developed which may decrease toxic effects of "megavitamin" treatments.

Project Description:

Objectives: The objective of the project is the innovative design of drugs used in age related diseases. Two classes of drugs have been studied. The first group studied are beta-adrenergic blockers, which are prominent cardiovascular agents. The objectives of this study are to prolong activity of these drugs and to diminish their side effects. The prolongation of drug activity was obtained by preparation of drug derivatives which produced an irreversible change in the tissue receptors for these drugs. The diminishment of side effects was obtained by changes in drug structure which decreased the penetration of modified drugs in the part of the body where the presence of the drug is not required. In another part of the project the water solubility of lipid soluble vitamins are increased by complexation with specially designed carriers; this process may be expected to decrease some of the toxic effects of these compounds.

Methods Employed: The study requires application of both chemical and pharmacological techniques. The synthetic chemical work is carried out in the section. The use of contracts for the production of starting materials for the syntheses has been requested. Pharmacological studies which use *in vitro* systems are also carried out in the laboratory of the section; those studies for which *in vivo* systems are necessary are carried out by arrangements with Key Pharmaceuticals.

Major Findings:

A. Beta-Adrenergic Antagonists. Drugs of this group are extensively used to treat hypertension, cardiac arrhythmias, and angina pectoris. Numerous studies with these drugs show that the drug's effects are due to a blockade of receptors for adrenergic hormones, which are located on the surface of cells. A new derivative of a widely used drug of this class, alprenolol, was prepared. This derivative, $AlmCOCH_2Br$, has the potential not only to block, but also to destroy the receptor for adrenergic hormones. $AlmCOCH_2Br$ was extensively characterized *in vitro* systems and *in vivo* on rats of different age. Considerable differences were found between the results on senescent and maturing rats. In senescent rats up to 81-91% of the beta-adrenergic receptors in hearts and lungs could be blocked in an irreversible way. These results were similar to those obtained in maturing animals. In senescent animals the receptor number remains depressed even after one month, while in maturing animals the number returns to normal after 200 or 650 hours in hearts and lungs, respectively. The results point to the possibility of using an irreversible blockade to perform "molecular surgery" in senescent organisms, i.e. to excise part of the receptors in a practically permanent manner.

B. Lipid Soluble Vitamins. These compounds, in addition to their obvious nutritional values, have further beneficial effects. Unfortunately to obtain a significant measure of these additional effects, doses which already elicit toxicity are required. Thus retinoids, which are derivatives of vitamin A, have been shown to reduce the incidence of lesions in epithelium induced by carcinogens. Work done in this program focuses on lessening the toxic effects of retinoids through transforming them to a water soluble form by complexation with solubilizing agents. Cyclodextrins, compounds obtainable in the processing of starch, were used as starting materials in the preparation of solubilizing agents. Substitution by alkyl groups in cyclodextrins yielded compounds which enabled the preparation of stable aqueous isotonic solutions that contained up to milligram amounts of retinoids per milliliter. The same solubilizing agents were found effective also in the preparation of stable aqueous solutions of vitamins D, E, and K.

Significance to Biomedical Research and Program of the Institute. Work in the section focuses on compounds which are heavily used in treatments for the elderly.

Treatment by antihypertensive agents, a group of drugs to which beta-adrenergic blockers belong, is required for about 31% of the elderly (W. E. Hale et al., J. Amer. Geriatr. Soc. 27: 374-377, 1979). The above described results on beta-adrenergic blockers suggest that irreversibly acting drugs may gain a role in geriatric pharmacology. The development of drugs which would need to be administered only weekly would be of advantage since the total amount of drug used would decrease and senescent organisms are known not to handle xenobiotics well. Furthermore compliancy with the prescribed therapy would be easier to control. Although the approaches to the problems taken by the section are academic, they are by no means impractical. Support by the pharmaceutical industry, in the form of a stipend for one postdoctoral fellow, testifies to the practicality of the approaches taken; and the fact that this sponsorship does not have strings attached testifies to the fact that the integrity of basic research has not been compromised.

The work on fat soluble vitamins is related to problems of aging as well. Transformation of epithelial tissues is known to be prominent in aging and derivatives of vitamin A have the potential to bring some improvements to this area. Furthermore there are views, often based on not fully convincing data, that antioxidants, i.a. vitamin E, may have a role in amelioration of aging related problems. Systematical work on the decrease of toxic effects of fat soluble vitamins may give a valuable opportunity to test these compounds at much higher doses and thus help to decide on the practical value of such treatments.

Proposed Course of the Project. Continuation of the work on the above projects may hopefully lead to the knowledge necessary for the design of useful drugs.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00047-11 LCMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Structure-Function Relationships in Hemoglobin and Erythrocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Rifkind	Research Chemist	LCMB NIA
OTHER:	J. G. Mohanty	Visiting Fellow	LCMB NIA
	T. Suda	Visiting Fellow	LCMB NIA (EOD 2/1/81)

COOPERATING UNITS (if any)

Johns Hopkins University

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Inorganic Biochemistry

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NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	2.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms involved in regulating the binding of oxygen to hemoglobin and the transport of oxygen to the tissues. The project also focuses on ways in which these functions are impaired and change with age. We have therefore studied the mechanisms involved in the oxidation of hemoglobin. Oxidation affects oxygen transport because it produces nonfunctional hemoglobin, which no longer binds oxygen. These studies have been extended to include an investigation of the stability of the entire erythrocyte and the erythrocyte membrane as well as other structure function relationships in membranes.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin, and the role of the protein in controlling this function. (2) To study the mechanisms for maintaining hemoglobin in its functional form. (3) To study the mechanisms involved in regulating the transport of oxygen to the tissues. (4) To elucidate age-related changes in the composition and functional properties of the erythrocyte and its membrane.

Methods Employed: Various preparative procedures are used to purify erythrocyte proteins and to separate various components of the erythrocyte. Visible, uv and atomic absorption spectroscopy, as well as gel electrophoresis, are used to analyze for various erythrocyte components. The oxygenation and oxidation of hemoglobin solutions and whole erythrocytes are investigated under various conditions with and without the addition of various substances. Binding of metal ions and other small substances to hemoglobin and other erythrocyte components are studied by equilibrium dialysis. Electron spin resonance is used to observe paramagnetic spin labels and Cu(II), and to detect changes in the mobility of membrane components.

Major Findings:

A. Dipolar broadening of membrane protein spin labels - a new probe of membrane mobility. ESR spectra of protein sulfhydryl spin labels show a mixture of two spectra. One corresponds to strongly immobilized labels and a second to weakly immobilized labels. By investigating the effect of temperature on erythrocyte and adipocyte membranes labelled at protein sulfhydryls, it has been possible to demonstrate the existance of a third class of sulfhydryl groups. It has been shown that this class of sulfhydryl spin labels generally have weakly immobilized signals which are partially broadened by the close proximity to other such sulfhydryl spin labels. At least on the erythrocyte by using a sulfhydryl cross-linking reagent, it has been possible to show that these pairs of sulfhydryls are generally at the interface between protein molecules. The signal from these pairs of labels is very sensitive to the distance between them, which can be altered by temperature or other membrane modifications. This probe is thus sensitive to relative mobility of membrane protein molecules, unlike the other spin label and fluorescent probes which monitor fluidity in the lipid bilayer.

B. Relationship between phosphorylation and the environment of sulfhydryl spin labels on the erythrocyte membrane. It has been postulated (Birchmeier and Singer, J. Cell. Biol., 73: 647 (1977)) that phosphorylation of spectrin induces polymerization of the spectrin-actin cytoskeleton which maintains the shape and deformability of the erythrocyte. We have shown that it is possible to produce membrane phosphorylation after reaction of sulfhydryl groups which inhibit the ability of the erythrocyte ghost to maintain its shape.

This finding is consistent with a recent publication which indicates that the phosphorylation can be uncoupled from shape changes (Patel and Fairbanks, J. Cell. Biol., 88: 430-440 (1981)). ESR studies on spin labelled membrane sulfhydryl groups further indicate that even under conditions where phosphorylation does not maintain the shape of the erythrocyte, a dramatic conformational change is observed which involves a decrease in the mobility of the sulfhydryl groups at the interphase between spectrin molecules.

C. Cholesterol In the Erythrocyte Membrane. We have previously found that as a function of age the ratio of cholesterol to phospholipid increases. It was also found that this increase produces a decrease in the rate of lysis which is related to a decrease in membrane fluidity. In order to further investigate erythrocyte cholesterol, we have studied the effect of the oxidation of cholesterol to cholesten-3-one by cholesterol oxidase. The conversion of cholesterol to cholesten-3-one increases both the rate of lysis and membrane fluidity. These effects may be due to the elimination of a possible H-bond between the OH group of cholesterol and phospholipids.

An important aspect of these studies is the dramatic difference between liposomes and cells. Thus in liposomes the cholesterol is all reacted within minutes, while under the same conditions the reaction with intact erythrocytes takes hours and has a very long lag.

These differences must be related to the organization and accessibility of cholesterol in intact cells. It is expected that this may be a valuable probe for changes in membrane organization as a function of age.

D. Spin Label Studies of Adipocyte Ghosts. In collaboration with Dr. George Roth of the Clinical Physiology Branch we have undertaken spin label studies of adipocyte ghosts in order to elucidate alterations in these membranes with age. Using sulfhydryl spin labels we have found a dramatic irreversible increase in the weakly immobilized signal at 37°C. This effect is diminished in the presence of proteolytic inhibitors and has been shown to be due to the proteolytic partial disruption of the ghosts which liberate 10-15% of the protein into the ghost supernatant. These alterations make it impossible to perform aging experiments using these labels.

However, we have begun a study of membrane lipid fluidity of 12 and 24 month old rat adipocyte ghosts using the stearic acid probes which dissolve into the phospholipid bilayer. These results suggest that the old ghosts are relatively more fluid at high temperatures but less fluid at low temperatures. This difference is due to a phase transition in the 10-15°C region which is much more pronounced in the 24 month old ghosts than the 12 month old ghosts.

E. Spin Label Studies of Rabbit Kidney Brush Border Membranes. In collaboration with Drs. Ada Elgavish and Shirley Hilden of the Laboratory of Molecular Aging we have undertaken studies on brush border membranes with the ultimate goal of relating the transport phenomena studied in the Laboratory of Molecular Aging to alterations of membrane fluidity. Studies on the effect of cholecalciferol (Vitamin D_3) indicate that it decreases fluidity of liposomes but increases the fluidity of brush border membranes, while 7-dehydrocholesterol decreases the fluidity of membranes and liposomes. This dichotomy was explained by a cholecalciferol induced increase in phospholipid content of the membrane when it is incubated with liposome containing cholecalciferol. This decreases the ratio of steroid to phospholipid which is expected to increase fluidity.

F. Mossbauer and Electron Spin Resonance Studies of Oxidized Hemoglobin. In collaboration with Dr. Abraham Levy of Johns Hopkins University, we have undertaken a study of iron in frozen hemoglobin solutions by Mossbauer and ESR techniques. These studies were undertaken in order to understand the perturbation of copper on hemoglobin which results in the oxidation of the iron.

We have found definitive evidence for a pair of new low spin iron species in the H_2O, hydroxide and to a lesser extent the fluoride complexes of methemoglobin. The relative amount of this new species is greatly enhanced by slow gradual freezing of hemoglobin. A direct perturbation due to the freezing process is, however, ruled out by the finding that this species can be formed from the usual high spin and low spin species even at 210°K where water is already completely frozen. While these species were detected at all pH's between 9 and 5 the relative amount of this species increases below pH 7. This pH dependence is similar to that found for a conformational equilibria which exists in solutions previously shown to be associated with the coordination of copper. By comparing ESR spectra at 11°K of the imidazole complex it was shown that the new species have g-values close to, though not identical to, those of the imidazole complex. Complexes with similar, though not identical, spectroscopic parameters are also obtained at 87% glycerol where it has been suggested that the H_2O in the sixth coordination site is replaced by the distal histidine. Some similar equilibria between the distal imidazole as a sixth ligand and the weak water and fluoride ligands may explain the presence of these species.

G. Interaction of Copper with Hemoglobin. Mossbauer studies on the copper bound to oxidized hemoglobin show that only the copper bound in the conformation of hemoglobin able to oxidize horse hemoglobin alters the Mossbauer spectrum of Fe while the other species has no effect on the Mossbauer spectrum of Fe. We are currently pursuing these studies in order to explain the nature of the perturbation of copper on the iron.

We have also been able to eliminate the high affinity human hemoglobin binding site by blocking the α-amino group with cyanate. This confirms our previous results which suggest that this site was in the region of the amino terminal of the β-chain of the hemoglobin molecule.

Significance to Biomedical Research and the Program of the Institute: The physiological role of hemoglobin is to transport oxygen from the lungs to the cells. The efficient uptake and release of oxygen requires cooperative oxygen binding and the proper regulation of the oxygen affinity. It is also necessary to maintain the integrity of the erythrocyte and to limit the oxidation of hemoglobin in order to maintain an adequate concentration of functional hemoglobin in circulation. These studies thus help to elucidate a vital function of organisms. The aging process can involve changes in the ability of the organism to transport oxygen to certain tissues. Studies on erythrocytes are also used to compare with and to help understand changes with age in the function and structure of other more complex cellular systems.

Proposed Course of the Project: (1) We plan to study the oxygenation of whole blood from individuals of various ages and try to correlate any observed changes with alterations in the erythrocyte composition and/or the hemoglobin molecule. (2) We plan to investigate the possible physiological significance of the reported zinc-induced increase in the oxygenation of erythrocytes. (3) We plan to further investigate the binding site for metal ions on hemoglobin and the mechanism whereby the bound metal ions alter the environment of the iron and thereby the functional properties of hemoglobin. (4) We plan to further study membrane fluidity and possible changes with age in the mobility of different membrane components of erythrocytes and other cells. (5) We plan to investigate the effect of membrane alterations on the rate of cholesterol oxidation in order to use this reaction as a probe for changes in membrane organization with age.

Publications:

Araki, K. and Rifkind, J.M.: Erythrocytes Membrane Cholesterol: An Explanation of the Aging Effect on the Rate of Hemolysis. Life Sciences 26: 2223-2230, 1980.

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Rifkind, J.M., Mohanty, J.G., Wang, J.T. and Roth, G.S.: A Reinterpretation of the ESR Spectra of Membrane Protein Spin Labels. Biophysical Discussions, in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00088-09 LCMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanisms of Cellular Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert E. Monticone	Biologist	LCMB NIA
	James T. Irving	Expert	LCMB NIA
	Gunther L. Eichhorn	Chief, LCMB	LCMB NIA

COOPERATING UNITS (if any)

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Laboratory of Cellular and Molecular Biology

SECTION
Section on Cellular Aging and Genetics

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of aging on cellular response to toxic levels of metal ions was studied. It was found that for several metals the relative toxicity to human cells was: Zn>Cu>Mn>Mg. Further studies with Zn and Mg revealed no significant difference in cellular toxicity with increasing passage levels (in vitro aging).

The effect of aging on sister chromatid exchange induction by fluorescent light was examined. Sister chromatid exchange levels were decreased in late passage human cells. It was also found that a low pretreatment dose of fluorescent light reduced SCE induction by later doses.

Studies on bone formation and aging have shown that the histological changes and the uptake of radio-Ca run parallel in young and old rats, being greatly retarded in old animals.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: (1) To determine the age related cellular effects of increasing metal ion concentrations. (2) To determine the relationship between cellular DNA repair capacity and *in vitro* aging. (3) To study age-related changes in bone metabolism, using an animal model.

Methods Employed: The BrdU-differential staining technique was utilized to measure DNA damage. Cell cloning and growth curve measurements were used to determine toxicity. An alkaline-elation technique is being developed to measure DNA repair.

The bone matrix implantation method originally described by Urist and later by Huggins and Reddi, has been used in the bone studies.

Major Findings:

1. The relative toxicity to human cells of the metal ions studied is: Zn>Cu>Mn>Mg.

2. There is no significant difference in the toxicity of Zn and Mg as a function of cellular passage level.

3. Sister chromatid exchange induction by fluorescent light was significantly diminished with increasing cellular passage level.

4. Lowering the Ca content of the diet fed to the young animals lowered the bone ash figure significantly, and also delayed the onset of calcification of the implants. This rather surprising result indicates that this calcification mechanism is not only controlled by the bone inducing factor (which is supposed to convert mesenchyme cells to osteoblasts), but also by the Ca content of the milieu. Increasing the Ca content of the diet fed to old rats had no effect in accelerating bone formation.

5. Preliminary experiments are under way to determine if the bone inducing factor is present in the bones of animals of all ages. It appears so far that this factor is not present in the bones of 24 month old rats. This may have important implications in the causation of osteoporosis.

Significance to Biomedical Research and to the Program of the Institute: The finding that various metal ions differ widely in strength of toxicity and appear to show no significant toxic difference upon human cells of varying passage adds to the knowledge of *in vitro* cellular growth and aging.

The finding that SCE induction by fluorescent light is diminished with increased cellular passage level is of particular interest due to the specific nature of fluorescent light damage.

The studies on bone formation indicate that mesenchyme cells which may have the potential to become osteoblasts and form bone, and are plentiful in young animals, are very scarce or absent in old rats; and also that these animals lack a specific bone inducing mechanism.

Proposed Course:

We intend to study the relationship between cellular aging and DNA repair capability. The effects of various metal ions on cellular DNA repair capability will be examined.

Publications:

Schneider, E.L., Monticone, R.E., Smith, J., Braunschweiger, K. and Roberts, T.: Skin Fibroblast Cultures Derived from Members of the Baltimore Longitudinal Study: A New Resource for Studies of Cellular Aging. Cell and Cytogenetics, in press.

Irving, J.T., LeBolt, S.A. and Schneider, E.L.: Ectopic Bone Formation and Aging. Clinical Orthopaedics and Related Research, 154: 249-253, 1981.

LABORATORY OF MOLECULAR AGING

The Laboratory of Molecular Aging has enjoyed a most successful and outstanding scientific year. For example, in this year, the Laboratory produced, *i.e.* published, in press or submitted, 55 research papers and major reviews, all placed in the highest quality, peer-reviewed, scientific periodicals. This has brought attention, credit and honor not only to the individual investigator, but also to the Gerontology Research Center, National Institute on Aging, as a locus for first-class, frontier-expanding, bio-medical research. Despite this pre-eminent record and performance, the outlook for the future looks gloomy, at best. Severe depletions due to attrition have been experienced in our scientific and supporting staff. The inability to obtain authorization to replace losses, if not to supplement staff, has left our research program seriously undercapitalized. As a consequence, auspicious opportunities are left awaiting and on-going programs representing considerable investment of time and resources are being threatened.

This report summarizes the following basic sciences projects: (A) Mechanisms of phosphate and calcium homeostasis, relation to senile osteoporosis and osteomalacia; (B) Pathophysiological and hormonal regulation of membrane transport systems; (C) Ion transport mechanisms; (D) Regulation of intermediary metabolism; and (E) Regulation of salivary gland function. In addition, the following clinical research project is described: (F) Studies on the oral physiological status of man during aging.

(A) Mechanisms of phosphate and calcium homeostasis, relation to senile osteoporosis and osteomalacia

Osteoporosis is a leading cause of mortality and morbidity in aging women. Last year, the Laboratory of Molecular Aging initiated a new project of research on the mechanisms of phosphate and calcium balance as part of a major effort to understand the pathophysiological bases of senile osteoporosis and osteomalacia. This investigation was prompted by our pioneering development of technology for the isolation of specific plasma membrane vesicles, the use of these isolated membrane vesicles to define transport mechanisms and by the findings that hormones, *i.e.* parathyroid hormone, calcitonin, and perhaps estrogen, prolactin and insulin; 1,25-dihydroxy Vitamin D_3; diet; and chronic uremic syndromes induce specific alterations in the activities of the membrane transporters that are reflected in changes in the absorption of phosphorus and calcium by the kidney and intestine.

The possible clinical use of Vitamin D_3 and its metabolites in postmenopausal osteoporotic women tends to obscure the fact that little is known about the function of Vitamin D and in particular about its biochemical mechanism of action. To provide this needed information, last year we established an experimental animal facility to produce Vitamin D-deficient chicks to be used as a model. The effect of Vitamin D status on the uptake of phosphate by kidney cells was examined. A new preparation of cells, isolated by a hyaluronidase-collagenase method, was developed and the cells were shown to be viable and metabolically active. The isolated cells accumulated phosphate and α-methyl-D-glucoside (a non-metabolizable sugar analog) in response to a Na^+ gradient. It was found for the first time that *in vivo* administration *i.o.* of 1 μg of 1,25-dihydroxy Vitamin D_3 to rachitic chicks effected within 3 hrs a 60% increase in the Na^+ gradient-dependent uptake of phosphate in the isolated cells. The specificity of the response was evident from the findings that the vitamin did not alter the uptake of phosphate in the absence of a Na^+ gradient, nor the uptake of the sugar in the presence or absence of the Na^+ gradient. This initial response to Vitamin D_3 administration to the animal also brought about a secondary alteration, an increase in the serum level of phosphate. With the resultant hyperphosphatemia, Na^+-dependent phosphate uptake by cells isolated 1 day after injection of the vitamin was inhibited.

The pioneering observation was made that 1,25-dihydroxy Vitamin D_3 incubated with kidney cells from D-deficient animals *in vitro* caused an increase in the Na^+-dependent phosphate uptake. The effect was seen within 1 hr and was concentration-dependent. A concentration as low as 10^{-14} M produced stimulation. Estimates from the uptake of the labeled vitamin indicated that, on the average, only 250 molecules of 1,25-dihydroxy Vitamin D_3 per cell was needed to stimulate uptake, and probably this value represented a considerable over-estimation. Cells from Vitamin D_3-repleted chicks did not respond to the vitamin when incubated *in vitro*.

The Vitamin D status of the animal was also reflected in the intestinal uptake of phosphate and calcium. For phosphate, cells isolated from the jejunum showed enhanced uptake (2-fold) after the *in vivo* administration of 1,25-dihydroxy Vitamin D_3. For calcium, cells of the ilium were most responsive.

It has been generally held that Vitamin D, like other steroid hormones, brings about its physiological effect by an interaction of the vitamin with intracellular receptors, transfer to the nucleus, and transcription of specific genes which code for transport proteins. Important initial experiments in our Laboratory now suggest an alternative mechanism, *i.e.* a direct effect of the vitamin on the membrane phospholipids, resulting in an altered permeability towards phosphate and calcium.

Another important determinant to the understanding of the role of Vitamin D in phosphate and calcium homeostasis is the regulation of the synthesis of the active form of the vitamin, 1,25-dihydroxy Vitamin D_3. Previous studies showed that the enzyme 1-hydroxylase which catalyzes the synthesis of the active form of the vitamin is localized in kidney mitochondria and its activity is controlled by various factors including serum levels of calcium, phosphate and parathyroid hormone as well as substrate, 25-hydroxy Vitamin D_3, and product, 1,25-dihydroxy Vitamin D_3. The mechanisms of regulation are unknown. Using isolated renal cells of D-deficient chicks and mitochondria derived from the cells it was found that the 1-hydroxylase was inhibited by 1,25-dihydroxy Vitamin D_3, *i.e.* the active form of the Vitamin D_3 regulated its own synthesis.

Studies were initiated on the mechanism by which the level of phosphate in the serum regulates renal phosphate transport. In the thyroparathyroidectomized rat, the acute infusion of phosphate increased the net tubular reabsorption of phosphate for 60 min but, thereafter, tubular reabsorption decreased. Glomerular filtration rate and urine flow were constant throughout. Importantly, this adaptation was reflected at the level of the brush border membrane. Brush border membrane vesicles, isolated from rats at the start of the infusion, accumulated phosphate at a rate of 1500 pmol/5 s·mg protein whereas uptake decreased to 880 pmol/5 s·mg protein after infusion. This parathyroid hormone-independent mechanism of regulating phosphate reabsorption by the kidney has great physiological and clinical significance and is being actively investigated.

A project was initiated to examine the mechanism by which calcium is transported transepithelially across the renal tubules. Thermodynamic considerations would suggest that the transport of Ca^{2+} from lumen to cell across the brush border membrane is down an electrochemical gradient and, therefore, mediated by a facilitated diffusion system. In contrast, movement of Ca^{2+} from cell to blood across the baso-lateral membrane is against its electrochemical gradient and, therefore, should require an active transport process. Using isolated baso-lateral membrane vesicles, prepared by a new technique (described below), a Ca^{2+}-Na^{+} antiport system was identified which would enable Ca^{2+} to be pumped out of the cell in exchange for Na^{+} entering down its electrochemical gradient. The effect of imposed membrane potentials indicated that the system was electrogenic, *i.e.* more than 2 Na^{+} were exchanged for each Ca^{2+}. The apparent Km values were 66 µM for Ca^{2+} and 15 mM for Na^{+}. The flux through the system was sufficiently great to suggest that this exchange reaction could represent the major pathway and driving force for energizing the reabsorption of Ca^{2+} in the kidney.

(B) Pathophysiological and hormonal regulation of membrane transport systems

A novel discovery, that glucocorticoids regulated specific transport systems in the kidney, was made. Previous studies had speculated that glucocorticoids could alter proximal tubular function for it was noted that glucocorticoids changed the acid-base status and net acid excretion of man and animals and also caused a parathyroid hormone-independent phosphaturia. These reports become explicable with our finding that glucocorticoids *in vivo* enhanced Na^{+}-H^{+} exchange in brush border membrane vesicles. The treatment groups were: I. sham operated; II. sham + high dexamethasone; III. adrenalectomy (ADX); IV. ADX + aldosterone; and V. ADX + maintenance dexamethasone. The activity of the Na^{+}-H^{+} carrier, measured by the uptake of $^{22}Na^{+}$ in the presence of a pH gradient ($pH_i = 5.5$, $pH_o = 7.5$), in groups I, III and IV were the same. Transport in group II was 70% greater than that in group I. Transport in group V was 50% greater than that in group III. These results indicated that glucocorticoids, but not mineralocorticoids, increased exchange activity. The Na^{+}-H^{+} exchange represents the major mechanism for acid secretion and bicarbonate reabsorption in the proximal tubule. The antiport system also represents a major pathway for Na^{+} reabsorption, independent of the co-transport systems. Thus, the glucocorticoid-enhanced exchange activity should be concomitant with a dissipation of the Na^{+} gradient driving force, resulting in a decrease in the activity of Na^{+}-cotransport systems in the presence of a Na^{+} gradient but not in the absence of the gradient. This was precisely found. Na^{+} gradient-dependent phosphate and D-glucose uptakes were inhibited in groups II and V. In the absence of the gradient, *i.e.* $Na^{+}_{i} = Na^{+}_{o}$, uptakes in all groups were the same. Thus, the previously observed phosphaturia is now explained.

It was known that in chronic uremia or in pathophysiological states in which nephrons are lost, such as occurs in aging, compensatory mechanisms are invoked to maintain phosphate homeostasis. The nature of these mechanisms is unknown and it represents one of the most active areas of investigation by renal and endocrine physiologists. Making use of the expertise inherent in the Laboratory of Molecular Aging, we recently initiated a project in collaboration with investigators at Baltimore City Hospitals and The Johns Hopkins School of Medicine to explore these compensatory mechanisms. The experimental animal model was the rat made uremic (BUN increased 4-times) by the removal of one kidney and the ablation of two-thirds of the remaining kidney. In important, but preliminary findings, we found that Na^{+} gradient-dependent phosphate transport was decreased. Thyroparathyroidectomy restored the activity. The uremic animal, however, still responded to a low phosphorus diet by increasing phosphate transport. Na^{+}-H^{+} exchange was not affected.

Parathyroid hormone increases phosphate excretion by inhibiting phosphate transport in the renal tubule. Although the mechanism by which the hormone inhibits transport is unknown, it was found that the action of the hormone, *in vivo*, was reflected in an alteration of the Na^+-phosphate co-transport system in the isolated brush border membrane of the proximal tubular cell. Further studies, *in vitro*, demonstrated that membrane vesicles, isolated from renal cortical slices pre-incubated with ^{32}P to label ATP, and then incubated with either parathyroid hormone or dibutyryl cAMP, had an altered uptake of phosphate and a modified state of protein phosphorylation. Na^+ gradient-dependent phosphate uptake was inhibited. The phosphorylation of 5 proteins having molecular weights of 170 K, 135 K, 105 K, 88 K, and 68 K were enhanced by the hormone or the cAMP analog. Phosphorylation of isolated membrane vesicles before and after disruption with detergent indicated whether the proteins faced the luminal or the cytosolic side of the membrane. Ca^{2+} also affected the phosphorylation of specific proteins.

Last year, we reported the establishment of a tissue culture facility and the use of cultured kidney cells to study transport mechanisms and their regulation. Na^+ gradient-dependent phosphate and sugar systems were characterized. The uptake of phosphate was found to be inhibited by parathyroid hormone and vasopressin, hormones which in these cells induced the synthesis of cAMP and increased protein kinase activity, but uptake was not appreciably affected by glucagon nor isoproterenol, hormones which in these cells did not significantly affect cyclic nucleotide metabolism. Initial studies indicated that Forscolin, a newly described potent activator of adenylate cyclase, inhibited phosphate transport and increased protein kinase activity markedly.

Last year, we reported on the age-dependent changes in the molecular structures and activity of the renal brush border membrane enzyme, maltase. In continuing studies we found that the enzyme from old (24 mo) had more (1.5 to 3.0-fold) antigenic sites per mg protein than the enzyme from mature (6 mo) animals. These results, together with earlier findings of the changes in enzyme conformation as a function of age, prompted us to try to develop monoclonal antibodies specific for the old and/or young molecules. Preliminary results were encouraging.

The technology of the production of monoclonal antibodies was adapted and was used as a means to resolve the difficult problem of the purification and characterization of specific renal brush border membrane carriers and how they might be changed during aging and in different pathophysiological states. Using other techniques, previous studies from several laboratories were not very successful due to the insolubility of the transport proteins in aqueous solution, their low frequency in the membrane, and the lack of an adequate assay for activity as the proteins were purified. In current studies, several cultures of fused spleen-myeloma cells produced antibodies that inhibited specific transport systems and, thus, proved to be strong candidates for cloning. Thus, promising leads were developed which need to be exploited.

A membrane preparation enriched in the baso-lateral segment of the plasma membrane was isolated from the rat renal cortex by a procedure that included separation of particulates on a self-generating Percoll gradient. The uptake of L-glutamate by the baso-lateral membrane vesicles was studied. A Na^+ gradient ($[Na^+]_o > [Na^+]_i$) stimulated the uptake of L-glutamate and provided the driving force for the uphill transport of the acidic amino acid, suggesting a Na^+ L-glutamate co-transport system in the baso-lateral membrane. A K^+ gradient ($[K^+]_i > [K^+]_o$) increased the uptake additionally. This effect was specific for $K^+(Rb^+)$. The action of the K^+ gradient in enhancing the uptake of L-glutamate had an absolute requirement for Na^+. In the presence of Na^+, but in the absence of a Na^+ gradient, *i.e.* $[Na^+]_o = [Na^+]_i$, the K^+ gradient also energized the concentrative uptake of L-glutamate. This effect of the K^+ gradient was not attributable to an alteration in membrane potential. The finding of a concentrative uptake system for L-glutamate energized by both Na^+ ($[Na^+]_o > [Na^+]_i$) and K^+ ($[K^+]_i > [K^+]_o$) gradients in the baso-lateral membrane, combined with previous reports of an ion gradient-dependent uphill transport system for this amino acid in the brush border membrane, suggests a mechanism by which L-glutamate is accumulated intracellularly in the renal proximal tubule to extraordinarily high concentrations.

The regulation of phosphatidylinositol turnover by α-adrenergic agonists in rat parotid acinar cell aggregates was examined with respect to kinetics and agonist-antagonist interactions. Phosphatidylinositol turnover was followed by the changes in the specific activities of ^{32}P-phosphatidic acid and ^{32}P-phosphatidylinositol. The specific activity of phosphatidic acid increased rapidly (within 1 min) after addition of epinephrine (10^{-5} M), reached a maximum level by 12 to 16 min, and then decreased. Incorporation of ^{32}P into phosphatidylinositol exhibited a lag phase of about 5 min and then increased continuously for an additional 40 min. The absolute amounts of phosphatidic acid and phosphatidylinositol did not change. The concentrations of epinephrine needed to stimulate ^{32}P incorporation into phosphatidic acid and phosphatidylinositol, measured at 15 and 30 min, respectively, were similar; K_a values of $2.05 \pm 0.46 \times 10^{-6}$ M for phosphatidic acid and $2.98 \pm 0.30 \times 10^{-6}$ M for phosphatidylinositol were found. The effects of agonists on ^{32}P labeling of phosphatidylinositol were, in order of potency, epinephrine≥norepinephrine>phenylephrine>>normetanephrine. When various adrenergic antagonists were evaluated for their ability to inhibit 10^{-5} M epinephrine-stimulated ^{32}P incorporation into both phosphatidic acid and phosphatidylinositol, the order of antagonist potency was prazosin≥phenoxybenzamine>phentolamine>yohimbine>>> propranolol. These findings indicate that phosphatidylinositol-phosphatidic acid turnover in the rat parotid gland is mediated by the α_1-adrenergic receptor system.

(C) Ion transport mechanisms

The age-dependent decrease in heart muscle calcium (Ca^{2+}) transport, previously reported, was examined further. In cardiac sarcoplasmic reticulum, Ca^{2+} transport is obligatory linked to ATP hydrolysis, a process that is rate-limited by breakdown of the phosphoenzyme intermediate. To determine if the age-dependent decline in V_{max} for Ca^{2+} transport is due to a decreased rate of phosphoenzyme breakdown, the turnover of phosphoenzyme was compared in the sarcoplasmic reticulum from old and young rat hearts. The turnover in the aged heart was 20% slower than that of the young heart ($p<.005$), a value that compares favorably with the decline in V_{max} for Ca^{2+} transport in old hearts.

A major pathway by which Ca^{2+} enters the cardiac cell is by Na^+-Ca^{2+} exchange across the sarcolemmal membrane. The mechanism of Na^+-Ca^{2+} exchange in cardiac sarcolemmal membrane vesicles was studied by stopped-flow spectrometry using arsenazo III as the Ca^{2+} indicator. The time-course of Na^+-dependent Ca^{2+} efflux from preloaded vesicles showed an initial linear release followed by a gradual decline in activity. The dependence of the efflux velocity on Na^+ concentration was sigmoidal with a Hill coefficient near 3, indicating that at least 3 Na^+ were involved in the activation of Ca^{2+} release. Vesicles loaded with Ca^{2+} by

ATP-driven uptake showed a higher affinity for Na^+ than passive- or exchange-loaded vesicles, an effect possibly resulting from phosphorylation of a component of the Na^+-Ca^{2+} exchange transport system.

The enzyme reactions catalyzed by the Ca^{2+}- and Na^+K^+ATPase were studied in greater detail by examining the kinetics of dephosphorylation of the phosphorylated enzyme by ADP. Sensitivity of the Ca^{2+}ATPase phosphoenzyme to millimolar Ca^{2+} appeared very shortly after the start of phosphorylation, indicating that translocation occurs very rapidly. The observed steady-state level of ADP-sensitive phosphoenzyme was much greater than the amount predicted from the forward and reverse rate constants for the translocation reaction, suggesting modification of the Ca^{2+}-dependent scheme for ATP hydrolysis. Dephosphorylation of the Na^+K^+ATPase by ADP resulted in the production of more inorganic phosphate than could be accounted for by phosphoenzyme decay. This finding suggested the existence of a pool of tightly bound ATP that was hydrolyzed by a parallel pathway.

(D) Regulation of intermediary metabolism

In further studies of the age-linked changes in the activity of enzymes involved in major catabolic pathways in heart, the Laboratory of Molecular Aging examined the effects of Ca^{2+} on the oxidation of pyruvates and the operation of the tricarboxylate cycle in isolated rat heart mitochondria. All three of the non-equilibrium (and thus rate-determining) steps, namely pyruvate dehydrogenase, NAD-isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase, were found to be controlled by micromolar concentrations of free-Ca^{2+}. Thus, there is a strong possibility that the concentration of Ca^{2+} in the cytosol, to which the mitochondria are exposed, may play an important role in controlling the final oxidative pathways by which carbohydrates and fats are degraded, and which release the majority of the energy needed by the cell.

The realization that Ca^{2+} control of dehydrogenase only occurred when the mitochondrial Ca^{2+} content was below 3 nmol/mg protein led us to characterize mitochondrial Ca^{2+} uptake and release over the range 1 to 30 nmol/mg. This was one or two orders of magnitude lower than the Ca^{2+} loads previously used. A saturable Ca^{2+} egress pathway having a $K_{0.5}$ of 10 nmol of intramitochondrial Ca^{2+} per mg of protein was described. This pathway was significantly less active in senescence. Rates of efflux were diminished about 30% in old age (24 mo vs. 6 mo) with a $p<0.01$. This decrement will have repercussions on the control of energy metabolism.

Age-linked changes in the activity of enzymes of the tricarboxylate cycle and lipid oxidation, and of carnitine content in muscles of the rat were found. The activities of citrate synthase, NAD-isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase were measured in homogenates of different muscles, as indicative of the potential capability of these muscles to carry out tricarboxylate cycle oxidations, as a function of age. In addition, several enzymes of fatty acid oxidation and the tissue content of carnitine were measured, as indicative of the capacity for lipid oxidation. The muscles chosen were heart, diaphragm (mixed red and white fibers), and soleus (mainly red fibers). There was a generalized decline in oxidative capacity in all three muscles with old-age (24 mo vs. 6 mo), this being greatest in the soleus and least in the heart. In addition, there was a specific deficiency in the ability to oxidize fatty acids, relative to other substrates, in heart muscle in senescence.

(E) Regulation of salivary gland function

Dispersed submandibular gland cells were utilized to evaluate new protein production and release. In these cells, as opposed to acinar cell preparations from the parotid gland, both α- and β-adrenergic receptor stimulation were required for maximum exocrine protein release. A broad size range of newly synthesized proteins was detected in soluble cell and media fractions from cell incubations ($M_r \sim 10^4$-5 x 10^5). Adrenergic stimulation (l-epinephrine) specifically increased the secretion of certain radiolabeled proteins, and, in addition, resulted in both cellular and secreted proteins with electrophoretic characteristics distinct from that of control preparations. Thus, adrenergic stimulation of these cells not only promoted secretion but profoundly affected protein processing as well.

Several descriptive characteristics of parotid glands from young adult (6 mo) and aged (23, 24 mo) male rats were determined. No differences in gland DNA content were found. However, a significant decrease in total protein content was observed (young = 17.3 mg/mg DNA, old = 14.3 mg/mg DNA). Also, the gland content of amylase was decreased approximately in half. Amylase activity in young animals = 3500 U/mg DNA and in old animals was 1876 U/mg DNA. Immunoreactive amylase from young animals was 11.4 mg/mg DNA while that in old animals = 6.1 mg/mg DNA. This suggests an alteration in secretory protein production (synthesis, degradation) in parotid glands from different aged animals.

α-Adrenergic receptors in rat parotid glands were characterized. With ^{3}H dehydroergocryptine as a radioligand, a heterogeneity in receptor subtype could be discerned by studying displacement of ligand with prazosin (relatively specific for α_1 sub-types) and yohimbine (relatively specific for α_2 sub-types). Using prazosin, 2 distinct populations of receptors were seen, in about equal proportions. The binding of [^{3}H]prazosin to parotid membranes was rapid, reversible, saturable and stereospecific, and showed a high affinity (K_D = 0.38 nM) and low capacity (V_{max} = 380 fmol/gm tissue). The characteristics of [^{3}H]prazosin binding were in good agreement with results from catecholamine stimulated K^+ release. This suggests that K^+ release from the rat parotid gland is an α_1-adrenergic mediated event.

When cells from young rats were pre-incubated with $^{45}Ca^{2+}$ and then exposed to 10^{-5} M l-epinephrine, a rapid rate of Ca^{2+} efflux was noted (∼6.0%/min). Similar studies with cells from aged rats showed a significant diminution (approximately 70% lower) in mobilization of cellular $^{45}Ca^{2+}$ pools. Such alterations in α-adrenergic responsivity were not due to changes in α-adrenergic receptor characteristics. Both the receptor number (19.8 fmol/mg protein, 3 mos; 32.7 fmol/mg protein, 12 mos; 33.3 fmol/mg protein, 24 mos) and affinity (K_D) (0.65 nM, 3 mos; 1.1 nM, 12 mos; 1.35 nM, 24 mos) did not change in a manner consistent with a receptor defect. The evaluation of α-adrenergic mediation of phosphatidylinositol (PI) turnover did however show an age-related decrement, consistent with alterations in K^+ and Ca^{2+} efflux. PI turnover in 24 mos rats was reduced about 45% ($p<0.01$) from that seen in young adult rats.

(F) Studies on the oral physiological status of man during aging

Several general dental characteristics of the Baltimore Longitudinal Study participants were evaluated. The number of decayed, missing or filled teeth (DMFT) increased with age among men, and with post-menopausal status among women. The total number of natural teeth present in subjects decreased with age [∼26 for young men (20-39 Y), ∿25 for middle-age men (40-59 Y), ∿20 for older men (≥60 Y); ∿26 for pre-menopausal women, ∿23 for post-menopausal women]. A strong correlation between age (or post-menopausal status, for women) and prevalence of cervical caries (total-active and filled) was noted; for men - 1.03, young; 2.21, mid-age; 4.2, old; for women - 0.8, pre-menopausal; 4.1, post-menopausal. Gingival health was evaluated and no differences were observed between any age or sex grouping. There was a strong relationship noted between age (or post-menopausal status) and the periodontal disease index. Older individuals showed significantly greater levels of periodontal diseases than their younger counterparts.

Exocrine protein secretion was evaluated following 2%-citrate stimulation of salivary flow from the parotid gland. The release of the acidic proline-rich proteins (PRP) into saliva was used as an index of this process. Data from 220 participants were utilized. Measurement of PRP was accomplished by a micro-immunodiffusion assay, with antisera specifically directed at the human PRP. No alterations, for both men and women, in the concentration of PRP in stimulated parotid saliva were observed across different age groups. Also the percentage of total protein, represented by the PRP (%PRP), remained constant with age (total protein content in saliva was also stable with age). These data suggested that exocrine protein secretion remains unaltered with age.

Data from over 120 subjects, on determined taste thresholds for each of the 4 basic taste modalities (sweet, sour, salty and bitter), were analyzed. Results with sodium chloride demonstrated a modest, but significant decrease in the ability of older persons (men and women) to detect threshold levels of this tastant (*i.e.* the threshold increases with age, $r = 0.38$, F ratio = 14.9, $p<.0005$). This change was gradual, occurring throughout the lifespan, rather than being a precipitous alteration only in the elderly. For quinine sulfate, a bitter taste, a lesser but also significant diminution in detection threshold ability occurred with increased age ($r = 0.25$, F ratio = 5.3, $p<.025$). The nature of this alteration was best described as gradual, like that with sodium chloride. The taste detection thresholds for both sweet (sucrose) and sour (citric acid) modalities were not significantly affected by age. However, a sex difference in the threshold for citric acid was observed (F ratio = 6.6, $p<.012$).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00041-07 LMA

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging I
Membrane Transport Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Combined into Projects Z01 AG 00051-01 LMA and Z01 AG 00052-01 LMA.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00042-07 LMA

PERIOD COVERED
October 31, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging II
Mechanisms of Hormonal Regulation

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Combined into Projects Z01 AG 00051-01 LMA and Z01 AG 00052-01 LMA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00043-08 LMA

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of Intermediary Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies seek to define and describe the molecular mechanisms whereby the major catabolic (*i.e.* energy-releasing) pathways of intermediary metabolism are controlled, and to investigate the ways in which these controls are altered in old-age. Such alterations are considered to underlie the decreased ability of senescent animals (including humans) to withstand environmental stresses (*e.g.* starvation, severe cold). Current investigations feature: (1) the control of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase activities by micromolar concentrations of Ca^{2+}-ions; (2) the transport of Ca^{2+}-ions into and out of heart mitochondria, at very low concentrations, and the impact of aging; (3) the control of pyruvate dehydrogenase in skeletal muscle and the effect of fatty acid oxidation; and (4) the measurement of the key rate-limiting enzymes of fatty acid oxidation and the tricarboxylate cycle in heart, diaphragm and soleus muscles, in young adult and senescent animals.

Project Description:

Objectives: These studies seek to define and describe the biochemical mechanisms which regulate the pathways of energy-transduction in animal tissues. Alterations, sometimes quite subtle, in these mechanisms are the most plausible cause of the decreased ability of older animals to adapt to environmental stresses. Currently, we are studying the interaction of fatty acid and carbohydrate oxidation at the level of pyruvate dehydrogenase in various muscle tissues, having previously shown that fatty acids are less actively oxidized by heart muscle mitochondria in old-age. If true of skeletal muscles, this would be important in limiting the ability of the older animal to spare glucose during starvation. Our studies also have the objective of delineating the role of Ca^{2+}-ions as a messenger between muscle contraction and the key energy-transducing systems of the mitochondrion. This involves the control of dehydrogenase activity by Ca^{2+}-ions, a field in the development of which this laboratory has played a large part.

Methods Employed: The experimental systems used include isolated intact mitochondria from heart and skeletal muscle, muscle homogenates and purified enzymes. Experiments involve the use of physical techniques (*e.g.* single and dual-wavelength spectrophotometry, polarography, fluorimetry, and the use of ion-specific electrodes) and also analysis involving enzymological methods.

Major Findings:

The Effect of Micromolar Concentrations of Ca^{2+}-ions on the Activity of Pyruvate Dehydrogenase and 2-Oxoglutarate Dehydrogenase Within Rat Heart Mitochondria. All three of the non-equilibrium (and thus rate-determining) steps in the oxidation of pyruvate and the operation of the tricarboxylate cycle have now been shown to be controlled by micromolar concentrations of free Ca^{2+}-ions. These steps are those catalyzed by pyruvate dehydrogenase, NAD-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. Thus there is the strong possibility that the concentration of Ca^{2+}-ions in the cytosol, to which the mitochondria are exposed, may play an important role in controlling the final oxidative pathways by which carbohydrates and fats are degraded, and which release the majority of the energy needed by the cell. This control might then supplement the control by ATP/ADP ratios, which is well understood to link together anabolic and catabolic processes.

In studying this postulated control mechanism, we related the mitochondrial content of active pyruvate dehydrogenase (PDH_A) to the amount of Ca^{2+} associated with rat heart mitochondria, as determined by atomic absorption spectrophotometry. Addition of very small quantities of the chelating agent EGTA allowed the stepwise withdrawal of mitochondrial Ca^{2+} until the PDH_A content began to diminish. Quantitative treatment revealed that the pyruvate dehydrogenase was 50% in the form PDH_A when the Ca^{2+} content was 1.0 nmol/mg protein. This is the first such direct measurement of the amount of intramitochondrial Ca^{2+} needed to activate any of the three mitochondrial dehydrogenases known to be Ca^{2+}-sensitive. The amount of Ca^{2+} required is 1 to 2 orders of magnitude lower than that normally used by investigators concerned with mitochondrial Ca^{2+} transport *per se*, and suggests that efforts should be redirected to these lower levels.

Somewhat similar experiments were performed using a low concentration of 2-oxoglutarate (0.5 mM) as substrate for isolated heart mitochondria, but here the activity of the dehydrogenase in its intramitochondrial milieu was followed by recording the fluorescence of mitochondrial NADH and NADPH. Control experiments involving the extraction of mitochondria followed by enzymatic analysis of the extracts revealed that the great majority of the fluorescence changes occurring in response to Ca^{2+} addition and withdrawal could be attributed to changes in NADH content. Again, enzymic activity was diminished to 50% by the removal of Ca^{2+} from the mitochondria until 1.0 nmol/mg of protein remained; at contents of 3 nmol/mg and greater, maximal activity was expressed. The incubation medium in these studies comprised 0.13 M KCl, 20 mM K Hepes, 5 mM KPi, pH 7.2. Addition of 1 mM $MgCl_2$ or omission of inorganic phosphate made the dependence of enzymic activity on Ca^{2+} content much steeper, presumably by lowering the intramitochondrial buffering capacity for Ca^{2+}-ions. The 50% activation at 1 nmol/mg of protein was unchanged, however.

The activity of 2-oxoglutarate dehydrogenase was also studied as a function of extramitochondrial Ca^{2+}-ion concentration. This was stabilized using $CaCl_2$:EGTA buffers and data are expressed in the form of pCa. It was found that 50% activation of 2-oxoglutarate dehydrogenase occurred at pCa 6.58, when the Na^+ concentration was 10 mM, the Pi concentration 5 mM and the added Mg^{2+} concentration was 1 mM. Omission of Pi resulted in 50% activation at pCa 6.77; omission of Mg^{2+} in 50% activation at $pCa \geq 7.3$. The effect of Pi is not well understood, but probably relates to intramitochondrial Ca^{2+} buffering; the effect of Mg^{2+} is probably due to inhibition of the inward, electrogenic transport of Ca^{2+} by the Mg^{2+}-ion.

The continuous recording of mitochondrial NADH fluorescence provided a means whereby rates of activation and inactivation of 2-oxoglutarate dehydrogenase could be followed, on the addition of $CaCl_2$:EGTA buffers and of excess EGTA, respectively. The rate of inactivation was dependent on the presence of Na^+ ions, with a $K_{0.5}$ of 2.5 mM, this presumably reflecting the egress of Ca^{2+} from the mitochondria by a $Ca^{2+}/2Na^+$ exchange carrier which has been described. It was also enhanced by the presence of Pi and Mg^{2+}, the mechanism of this being ill-understood.

Mitochondria isolated from the hearts of senescent (24 months old) rats showed an undiminished rate of dehydrogenase activation on adding $CaCl_2$:EGTA buffers, but a rate of inactivation by excess EGTA that was diminished by 40% ($p<0.005$). This suggested a decreased $Ca^{2+}/2Na^+$ transport activity in these mitochondria in old age, and this was investigated directly (below). In addition, there was a modest shift with old-age in the curve relating the steady-state percentage reduction of mitochondrial NAD to the pCa of the medium: this is consistent with a greater decline with old age in Ca^{2+}/Na^+ transport activity (the egress pathway) than in the electrophoretic movement of Ca^{2+}-ions (the entry pathway). Control experiments with Triton-solubilized mitochondria showed that there was no change with old-age in the Ca^{2+}-sensitivity of the 2-oxoglutarate dehydrogenase enzyme *per se*. These age-related changes in the Ca^{2+}-transport properties of heart mitochondria, which are also described below from a different perspective, suggest first that the degree of activation of pyruvate dehydrogenase and the tricarboxylate cycle will be greater at a given cytosol pCa in the senescent animal and second that any beat-to-beat variations in intramitochondrial Ca^{2+} concentration will be muted in old-age. This will then have repercussions on the control of energy metabolism, via the mechanisms outlined above.

The Uptake and Release of Ca^{2+} Ions by Heart Mitochondria at Very Low Total Ca^{2+} Loads: Impact of Senescence on These Processes. The realization that Ca^{2+}-ion control of dehydrogenases only occurs when the mitochondrial Ca^{2+}-ion content is below 3 nmol/mg protein led us to characterize mitochondrial Ca^{2+}-ion uptake and release over the range 1-30 nmol/mg. This is one or two orders of magnitude lower than the Ca^{2+} loads usually used in mitochondrial studies. We measured Ca^{2+} movements directly using the metallochromic indicator Arsenazo III and dual-wavelength spectrophotometry. After a phase of Ca^{2+} uptake, release was initiated by the addition of NaCl and Ruthenium Red (the latter to block re-entry). The rate of egress was dependent on the amount of Ca^{2+} which had been accumulated, with a $K_{0.5}$ of approximately 10 nmol/mg. Rates of Ca^{2+} efflux were diminished by 25%, 29% and 34% in old-age (24 mo versus 6 mo, M) at mitochondrial Ca^{2+} loads of 42, 33 and 11 nmol/mg of protein, respectively. When the Ca^{2+} load was smaller, only 5 nmol/mg including endogenous Ca^{2+} retained by the mitochondria during preparation, the decrement with old-age was larger, being 37%. All decrements were statistically highly significant ($p<0.01$). For these studies, glutamate plus malate were present as oxidizable substrates, to energize Ca^{2+} uptake. Control studies showed an unchanged rate of glutamate plus malate oxidation in old-age. Thus, the decreases in transport activity with age reflect changes in the transport system *per se*. Their possible biological significance has been touched on above.

Age-linked Changes in the Activity of Enzymes of the Tricarboxylate Cycle and Lipid Oxidation, and of Carnitine Content, in Muscles of the Rat. Although there have been many studies in which mitochondrial enzyme activities have been measured as a function of aging, almost all of these have focussed upon enzymes which catalyze near-equilibrium reactions and which cannot therefore exercise metabolic control, *e.g.* succinate dehydrogenase. By contrast, citrate synthase, NAD-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase all catalyze non-equilibrium reactions and can therefore exert control over flux through the tricarboxylate cycle, quantitatively the most important pathway in energy-metabolism. These activities are therefore indicative of the potential capability of muscle to carry out tricarboxylate cycle oxidations and were accordingly measured as a function of age. In addition, we measured acyl-CoA dehydrogenase activity, as potentially rate-limiting for fatty acid oxidation: carnitine content and carnitine acyltransferase activity were also measured, as we had previously shown them to decline in old-age in concert with the ability to oxidize fatty acids, in a study involving isolated heart mitochondria. The muscles chosen were heart, diaphragm (mixed red and white fibers) and soleus (mainly red fibers) and the experiments were carried out with whole-tissue homogenates. There was a generalized decline in the activity of all oxidative enzymes measured, and in the content of carnitine, this being greatest in the soleus and least in heart muscle. Heart muscle showed an additional specific decrease in the ability to oxidize fatty acids, as measured by 3-hydroxyacyl-CoA dehydrogenase and carnitine content.

We maintain that studies of this type can describe the potential activity of metabolic pathways with an economy of laboratory time and effort and identify changed patterns of metabolism in old-age. One such change is the lesser relative capability for fatty acid oxidation in heart described above for homogenates and in a previous Annual Report for isolated mitochondria.

Significance to Biomedical Research and to the Program of the Institute: The interaction of Ca^{2+}-ions with heart mitochondria forms part of the response of energy metabolism to changes in work-load on the heart. Information from this study is extremely relevant to studies by the Cardiovascular Section, CPB, on age-linked changes in the response of the heart to catecholamines and to work by the Biophysics Section, LMA, on age-linked changes in Ca^{2+}-ion transport by sarcoplasmic reticulum from heart. Clearly there is a multi-facetted disturbance of Ca^{2+}-ion homeostasis in the aging heart cell. The studies on the interaction of fatty acid and carbohydrate (pyruvate) metabolism provide a basis of information against which age-linked changes can be viewed. Age-linked decrements may limit the ability to tolerate starvation (see above).

Proposed Course of the Project: We will investigate pyruvate dehydrogenase interconversion in skeletal muscle mitochondria and the effect of fatty acid and ketone oxidation upon this process. There is very little work on the control of pyruvate dehydrogenase in skeletal muscle. The effectiveness of fatty acid oxidation in inactivating pyruvate dehydrogenase will have a major impact on the sparing of glucose in starvation. In the long term we intend to make comparisons between mitochondria from young and old animals, in terms of the effectiveness of this control mechanism.

We intend to pursue investigations on the degree to which mitochondria buffer the free $[Ca^{2+}]$ of their environment at the very low total Ca contents which we believe exist in mitochondria *in situ*. We expect that at 1 nmol Ca^{2+} per mg of protein (the amount which we have identified as giving 50% activation of the tricarboxylate cycle dehydrogenases) the buffering will be much less complete than that described in the literature. In other words, we believe that previous studies may have led to erroneous conclusions on mitochondrial physiology, through the use of Ca^{2+}-loads 10 to 1000 times greater than physiological. The question is the basic one of whether mitochondria control the cytosolic free Ca^{2+}-ion concentration, the orthodox view, or merely react to it.

Publications:

Hansford, R. G.: Control of mitochondrial substrate oxidation. In Sanadi, D. R. (Ed.): Current Topics in Bioenergetics, Vol. 10. New York, Academic Press, 1980, pp. 217-278.

Hansford, R. G.: Effect of micromolar concentrations of free Ca^{2+}-ions on pyruvate dehydrogenase interconversion in intact rat heart mitochondria. Biochem. J. 194: 721-732, 1981.

Hansford, R. G.: Review on bioenergetics and aging. In Florini, J. R. (Ed.): Chemical Rubber Company Handbook on the Biology of Aging, Biochemistry, Vol. 1. Boca Raton, CRC Press, in press.

Hansford, R. G. and Castro, F.: Age-linked changes in the activity of enzymes of the tricarboxylate cycle and lipid oxidation, and of carnitine content, in muscles of the rat. Mech. Ageing Dev., in press.

Hansford, R. G. and Castro, F.: Effect of micromolar concentrations of free Ca^{2+} ions on the reduction of heart mitochondrial NAD(P) by 2-oxoglutarate. Biochem. J., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00048-07 LMA

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Ion Transport Mechanisms.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any) Laboratory of Neurochemistry, National Institute of Neurological and Communicative Disorders and Stroke, NIH; Departments of Medicine, Pharmacology, and Physiology, University of Texas; Department of Biology, Johns Hopkins University.

LAB/BRANCH
Gerontology Research Center, Laboratory of Molecular Aging

SECTION
Biophysics and Intermediary Metabolism Sections

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

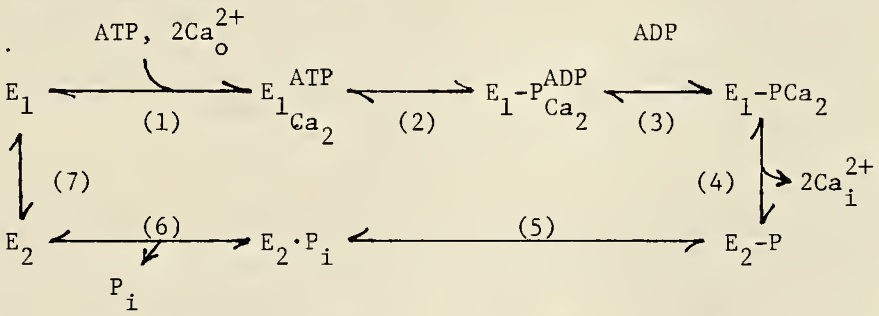
Kinetic studies on ATP- and Na$^+$-dependent ion transport mechanisms were carried out. The intermediate steps in the overall catalytic reaction mediated by the Ca^{2+}-ATPase and Na$^+$+K$^+$ ATPase enzymes were examined with respect to the dephosphorylation of the phosphoenzyme by ADP. The phosphoenzymes in the sarcoplasmic reticulum from hearts of aged rats turn over more slowly than did the enzyme from young animals. The rapid kinetics of the Ca^{2+}/Na$^+$ exchange reaction, which mediates the uptake of Ca^{2+} across the sarcolemma, was described. The mechanism of ATP synthesis in subchloroplast particles was clarified.

Objectives: (1) To characterize the enzymatic reactions linked to the active transport of metal ions catalyzed by the Na+K- and Ca^{2+}-activated adenosine triphosphatases. (2) To elucidate the mechanism of energy coupling between the energy producing enzymatic reactions and energy-utilizing transport reactions. (3) To elucidate the functional characteristics of Na^{+}/Ca^{2+} exchange. (4) To further characterize the changes in cardiac SR transport function associated with prolonged relaxation in aging myocardium.

Methods Employed: The rapid mixing acid-quench technique was used to monitor the early time course of the Ca^{2+}- and Na+K-ATPase intermediate reactions. Phosphoenzyme formation was followed by the consecutive addition of radiolabeled ATP and strong acid to the enzyme mixture with a variable time of incubation between the two additions. Decomposition of the phosphoenzyme was measured by adding ADP or EGTA to the phosphorylated enzyme and allowing it to decay for variable short time intervals before adding acid to stop the reaction. The time course of Na^{+}-dependent Ca^{2+} efflux in cardiac sarcolemmal vesicles was studied by dual-wavelength spectrophotometry combined with stopped-flow mixing using Arsenazo III as Ca^{2+} indicator. The ATPase and Ca^{2+} efflux reactions were parametrized and tested for conformity with various models by computer simulation.

Major Findings:

Kinetic Studies of the Ca^{2+}- and Na^{+}+K^{+}-ATPases: Dephosphorylation of the Phosphoenzyme by ADP. The hydrolysis of ATP by the CaATPase of sarcoplasmic reticulum involves the following steps:



Binding of Ca^{2+} to high affinity sites on the enzyme activates the formation of a phosphoenzyme (E_1-P) that is sensitve to ADP (*i.e.* reacts rapidly with ADP to form ATP). This step is followed by a conformational transition which converts the ADP-sensitive phosphoenzyme (E_1-P) to an ADP-insensitive form (E_2-P). Concomitant with or immediately following the phosphoenzyme interconversion (believed to be the translocation step) Ca^{2+} is released from the enzyme. The ADP-insensitive phosphoenzyme may either be hydrolyzed to E_2+P_i or, when the intravesicular Ca^{2+} concentration is sufficiently high (mM), be converted back to E_1-P by reversal of step 4. An analogous set of reactions can be written for the Na^{+}+K^{+}-ATPase.

Studies of the reaction of the Ca^{2+}ATPase phosphoenzyme with ADP were begun in 1979 and have been continued throughout the present fiscal year. Results of the previous years studies which are pertinent to the present findings are: (1) The time course of phosphoenzyme decay following the addition of ADP was resolved into rapid (>200 s^{-1}, 45% of total E-P) and slow (35 s^{-1}, 50% of total E-P) phases. (2) P_i

production was observed only during the slow phase and was less than stoichiometric with the slowly decaying E-P. (3) The rapid phase of phoephoenzyme decomposition corresponds to the combination of ADP with E_1-P and the formation of EATP (steps 2 + 3) while the slow phase corresponds to the breakdown of E_2-P which occurs both in the forward ($E_2\text{-P} \rightarrow E_2 + P_i$) and backward ($E_2\text{-P} \rightarrow E_1\text{-P}$) directions. The latter accounts for the less than stoichiometric release of P_i during the slow phase of E-P decay.

Conversion of E_2-P to E_1-P will occur when the intravesicular Ca^{2+} level becomes sufficiently high to allow Ca^{2+} to bind to low affinity sites on E_2-P. Accordingly, when the phosphoenzyme was reacted with ADP and Ca^{2+} sufficient to saturate the low affinity binding site, the rate of decay of the slow component increased (75 s^{-1}) reflecting a more rapid conversion of E_2-P to E_1-P. Lowering the internal Ca^{2+} by making the vesicles permeable to Ca^{2+} had the opposite effect (17 s^{-1}). As expected, P_i production was less in the former case than in the latter. The dependence of the rate of decay of the slow component on Ca^{2+} concentration had a Hill coefficient near 2 and a half saturation point between 1 and 2 mM Ca^{2+}. These results are consistent with the view that the Ca^{2+} site that is responsible for the increased rate of E_2-P decay is a transport (discharge) site.

In order to determine how rapidly Ca^{2+} was being transported from the outside of the vesicle to the inside, we examined the Ca^{2+} sensitivity of the rate of decay of the slow component as a function of the time of incubation with ATP. After only 6 msec of phosphorylation all of the slowly decaying E-P (accounting for 70% of the total E-P) was sensitive to Ca^{2+}. This implies that all of the steps up to and including the translocation reaction (step 4) are very fast (200-300 s^{-1}). Since reversal of the translocation step is comparatively slow (∿0-60 s^{-1} depending on whether or not a Ca^{2+} gradient is present) very little of the phosphoenzyme should be sensitive to ADP in the steady state. This contrasts with our experimental result showing that almost one-half of the steady state phosphoenzyme is sensitive to ADP (present as E_1-P).

In order to account for the discrepancy between the predicted and observed levels of E_1-P in the steady state we proposed the following scheme in which the enzyme functional unit is a dimer:

$$E_1/E_1 \underset{(1)}{\overset{ATP}{\rightleftharpoons}} E_1ATP/E_1 \underset{(2)}{\overset{ATP}{\rightleftharpoons}} E_1\text{-P}/E_1ATP \underset{(3)}{\overset{Ca^{2+},\ ADP}{\rightleftharpoons}} E_2\text{-P}/E_1\text{-P} \underset{(4)}{\rightleftharpoons} E_2{\cdot}P/E_1\text{-P}$$

Steps 1-3 are very fast and account for the rapid appearance of E_2-P (Ca^{2+}-sensitive slowly decaying E-P). The last step is a comparatively slow step and results in accumulation of $E_2\text{-P}/E_1\text{-P}$ in the steady state. This would explain why the phosphoenzyme in the steady state is about evenly distributed between rapidly (E_1-P) and slowly (E_2-P) decaying forms.

The reaction of the Na^++K^+-ATPase phosphoenzyme with ADP was studied in the presence of oligomycin which blocks the conversion of the ADP-sensitive phosphoenzyme (E_1-P) to the ADP-insensitive form (E_2-P). Addition of 5 mM ADP to the phosphorylated oligomycin-treated enzyme produced a biphasic pattern of dephosphorylation in which 2/3 of the phosphoenzyme decayed rapidly (>200 s^{-1})

and the remainder slowly (6 s^{-1}). Quantitatively similar results were obtained in the absence of oligomycin except that the slow phase decayed more rapidly (18 s^{-1}). Subtraction of a stable E-P component (which is not in the main catalytic pathway) from the total E-P revealed that more than 90% of the uninhibited enzyme is sensitive to ADP. These results indicate that in the native enzyme E_1-P accumulates in the steady state because E_2-P hydrolysis is much faster than the conversion of E_1-P to E_2-P. In the oligomycin-treated enzyme both E_2-P hydrolysis and the conversion step must be inhibited in order to allow the ADP-insensitive intermediate to build up to the comparatively high levels observed in the steady state.

We have also examined P_i production under conditions preventing rephosphorylation of the enzyme. When an excess of unlabeled ATP was added to the phosphoenzyme, P_i production was 4-5 times greater than the amount of phosphoenzyme initially present. A similar result was obtained when ADP was used to dephosphorylate the phosphoenzyme. Raising the ADP concentration had no affect on the rate or amount of extra P_i production.

The observation that under conditions where rephosphorylation was prevented more P_i was produced than could be accounted for by E-P decay suggested that there exists a pool of tightly bound ATP which continues to be hydrolyzed after binding of labeled ATP had been inhibited by the addition of excess ADP or unlabeled ATP. Consistent with this we found that when we measured the kinetics of ATP dissociation in a "commitment" experiment, the nucleotide dissociated biphasically (60% fast, 40% slow) with a final rate of $\sim 6\ s^{-1}$. The pool of tightly bound ATP is assumed to be in equilibrium with the weakly bound ATP and the phosphorylated enzyme as shown below:

$$E_1\text{+ATP} \rightleftharpoons \underset{\substack{\text{weak}\\ \text{complex}}}{E_1\text{ATP}} \rightleftharpoons \underset{\substack{\text{tight}\\ \text{complex}}}{E_1\text{'ATP}} \rightleftharpoons E_1\text{-P+ADP}$$

When this scheme was used to simulate the time course of E-P formation and P_i production the predicted level of the tight complex was much lower than expected from the amount of P_i production following dephosphorylation by either ATP or ADP. This raises the possibility that hydrolysis of the two pools of ATP takes place by parallel pathways (separate halves of dimer?).

Decomposition of the Phosphoenzyme in Sarcoplasmic Reticulum (SR) Prepared from Young and Old Rat Hearts. Isolated vesicles of SR show an age-dependent decrement in the ability to accumulate Ca^{2+} in the presence of oxalate. Our previous studies showed that the decrease in Ca^{2+} transport activity was not due to the following: (1) increased passive leak, (2) decreased transport site density, or (3) differential contamination by other membranes. Since V_{max} for Ca^{2+} transport in SR was decreased with age but the site density was unchanged, the only other possibility is that the rate-limiting step in the transport mechanism is slowed. In order to test this possibility, we measured the spontaneous decay of the phosphoenzyme in the presence of EGTA (added to prevent rephosphorylation of the enzyme). This reaction was chosen because it is rate-limiting in the overall ATPase reaction which, in turn, is required for Ca^{2+} transport. Following the addition of EGTA, 59% of the total phosphoenzyme in SR prepared from young hearts decayed rapidly as compared to 54% in the old hearts (p NS). When the rates of the rapidly decaying fraction of E-P were compared, phosphoenzyme in old hearts turned over more slowly ($t_{1/2} = 1.20 \pm .04$ sec) than phosphoenzyme in young hearts ($t_{1/2} = 1.03 \pm .03$ sec; $p<.005$). The percent difference in the rate of phosphoenzyme turnover in young and old SR is similar to the difference in V_{max} for Ca^{2+} transport (∿25%) supporting the view that in old hearts the decline in Ca^{2+} transport activity is the result of a decreased rate of phosphoenzyme turnover.

Mechanism of ATP Synthesis by Subchloroplast Particles. Chloroplast particles in plant cells, like mitochondria in animal cells, contain the machinery for synthesizing ATP, the primary energy-yielding substrate in biological systems. The mechanism by which chloroplasts are able to synthesize ATP from ADP and P_i is controversial. In one view (Roy and Moudrianakis) AMP is the primary phosphate acceptor and ATP is produced by the transfer of P_i from a donor to an acceptor ADP molecule as shown in the following scheme:

$$(1) \quad AMP \xrightleftharpoons{Pi^*} ADP^* \xrightleftharpoons[ADP]{ADP} ADP^* \rightleftharpoons AMP + ATP^*$$

In an alternate view of the mechanism (Boyer) ATP^* is produced by the combination of P_i^* with ADP:

$$(2) \quad ADP \xrightleftharpoons{P_i^*} ATP^*$$

Support for the first mechanism has come from steady state kinetic studies in which accumulation of ADP^* was demonstrated under conditions where ATP synthesis was taking place. The main objection to this mechanism is that chloroplast preparations contain adenylate kinase which can catalyze the formation of ADP^* from newly synthesized ATP^*:

$$2ADP \rightleftharpoons AMP + ATP$$
$$AMP + ATP^* \rightleftharpoons ADP + ADP^*$$

In order to distinguish between pathways (1) and (2) rapid mixing acid quench experiments were carried out in which the time course of formation of ADP^* and ATP^* were simultaneously measured under a variety of conditions. In each and every case, ADP synthesis lagged behind the formation of ATP. Further studies revealed that the low but significant levels of adenylate kinase present in subchloroplast particle preparations were responsible for the formation of ADP^* from newly synthesized ATP^*. These results indicate that the primary phosphate acceptor in ATP synthesis by plant chloroplasts is ADP (pathway 2) and that the low levels of labeled ADP found in chloroplasts are due to contaminating adenylate kinase.

Na^+-Ca^{2+} Exchange in Cardiac Sarcolemmal Vesicles. Regulation of the intravesicular Ca^{2+} level in cardiac tissue is believed to involve Na^+-Ca^{2+} exchange, a mechanism which couples the movement of 3 Na^+ in one direction with 1 Ca^{2+} in the opposite direction. The unequal movement of charge across the membrane with each cycle of the pump results in an accumulation of charge which can be prevented by increasing the membrane conductivity to cations using ionophores. We have studied the kinetics of Na^+-dependent Ca^{2+} efflux in preloaded cardiac sarcolemmal (SL) vesicles using dual-wavelength stopped-flow spectrophotometry and the Ca^{2+} indicator Arsenazo III. SL vesicles were loaded with Ca^{2+} by passive diffusion, by Na^+-Ca^{2+} exchange, or by ATP-driven Ca^{2+} uptake. When SL vesicles suspended in a medium containing 160 mM KCl were mixed with a solution containing 160 mM NaCl, a rapid initial phase of Ca^{2+} efflux lasting 1-2 seconds was observed followed by a steady decline in rate. Pretreatment of the vesicles with valinomycin, which creates an inside negative K^+ diffusion potential, increased the duration of the initial phase of Ca^{2+} efflux while replacement of Cl^- with the impermeant anion gluconate produced the opposite effect in addition to slowing the efflux rate. These results suggest that the initial decline in the efflux rate is due to the accumulation of positive charge resulting from the electrogenic nature of the exchange process.

The velocity of Ca^{2+} transport showed a sigmoid dependency on NaCl concentration with a Hill coefficient near 3 indicating that activation of Ca^{2+} release involves a minimum of three Na^+ sites. For vesicles loaded by passive equilibration or by Na^+-Ca^{2+} exchange half-maximal activation by NaCl occurred at 31 mM while for vesicles loaded by ATP-driven Ca^{2+} uptake the corresponding value was 22 mM reflecting an increase in the affinity of Na^+ sites under these conditions. The decreased threshold for Na^+ observed when ATP is used to load the vesicles could be due either to an effect of ATP on the Na^+-Ca^{2+} exchange process (protein-kinase dependent phosphorylation?) or to selective loading of a particular subpopulation of vesicles (presumably inside-out) during Ca^{2+} uptake.

Significance to Biomedical Research and to the Program of the Institute. Elucidation of the transport pathways involved in the control of muscle contraction and in the regeneration of ionic gradients necessary for the conduction of electrical impulses will provide insight into the mechanism(s) by which cells convert the chemical energy of phosphate-containing compounds into osmotic work. This information will be helpful in identifying biochemical alterations in function underlying physiological changes associated with age and disease.

Proposed Course of the Project. Experiments are planned to determine whether the mechanism of Na^+-Ca^{2+} exchange in cardiac sarcolemmal vesicles involves a consecutive or simultaneous movement of Na^+ and Ca^{2+} across the membrane barrier. The reactions of the SR pump with Ca^{2+} will be studied using phosphorylation to monitor the interaction of the enzyme with Ca^{2+}. We will attempt to phosphorylate the β (non-catalytic) subunit of Na^++K^+-ATPase using protein kinase from Ehrlich ascites tumor cells in order to have a model system in which to study the effects of β subunit modification on α (catalytic) subunit function.

Publications:

Hobbs, A. S., Froehlich, J. P. and Albers, R. W.: Inhibition by vanadate of the reactions catalyzed by the Na^+- and Na^+ plus K^+-stimulated ATPases. A transient state kinetic characterization. J. Biol. Chem. 255: 5724-5727, 1980.

Reeves, J., Trumble, W., Sutko, J., Kadoma, M. and Froehlich, J.: Calcium transport mechanisms in cardiac sarcolemmal vesicles. In Bronner, F. and Peterlik, M. (Eds.): Calcium and Phosphate Transport Across Biomembranes, New York, Academic Press, in press.

Sumida, M., Wang, T., Mandel, F., Schwartz, A. and Froehlich, J.: Transient state kinetics of cardiac muscle Ca^{2+}-ATPase. In Packer, L. and Mukohata, Y. (Eds.): Cation Flux Across Biomembranes. New York, Academic Press, 1979, pp. 101-102.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00049-03 LMA

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on the Oral Physiological Status of Man During Aging.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. M. Weiffenbach	Research Psychologist	CID, NIDR
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LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Intermediary Metabolism Section

INSTITUTE AND LOCATION

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1.5	1.1	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There has been little systematic investigation of tissues within the aging oral cavity. The purpose of this project is to assess the oral physiological status of participants in the Baltimore Longitudinal Study and thus provide baseline information which is lacking. The objectives of the study remain the same: (1) define subjects' dental and oral health; (2) examine biological factors likely to influence oral health. After three years, 310 subjects have been seen and approximately 150 pieces of information accumulated/subject. Major effort has been directed in collating data and analyzing results in three information areas: a) demographic and general dental descriptions of the subset of BLS subjects participating in the oral physiology study; b) evaluation of exocrine protein secretion from stimulated parotid glands; c) evaluation of taste acuity and taste intensity perception.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: This project is designed to describe oral and dental tissues during aging and evaluate physiological and pathological factors likely to influence these tissues. Much of our present knowledge regarding oral health in the aging individual comes from studies using institutionalized persons or persons whose general health status has been incompletely characterized. Participants in this study, as a component program of the Baltimore Longitudinal Study, are well characterized with respect to a large number of medical, physiological, psychological and sociological factors. The influence of such factors on oral health, as well as the influence of specific oral biological variables, are examined. Participants in this study will be seen once every 4 years in "two alternating protocols." It is hoped, in this way, that we can better define oral health throughout adult life and be able to predict the sequelae of various factors (disease, psychological stress, medications, etc.) on oral functions.

Methods Employed: All methods described following the first and second year reports on this project are still utilized. These include the history, clinical oral and dental examinations, radiographic examination, evaluations of whole and parotid saliva and dental plaque, evaluation of taste acuity (thresholds) and perception of taste intensity, and examination of tongue function during normal oral activities (speech, swallowing) using ultrasound methodology. No new methodologies have been introduced during this year.

Data storage and retrieval systems, and analyses of data with SPSS-prepackaged programs, are actively utilized. Update, verification and analysis of selected data on a frequent (usually weekly) basis are performed so that trends in findings, or problem areas, are detected routinely.

Major Findings: Since subjects participating in the oral physiology study represent only a subset of the BLS population (approximately 1/3), detailed demographic and social characterization was performed. In addition, several dental characteristics were examined. The subjects in the oral physiology study are typical of BLS participants: generally middle to upper-middle socioeconomic class, well educated, caucasians residing overwhelmingly (80%) in the greater Washington, D.C.-Baltimore area. Most (80-90%) receive regular dental care and practice similar oral hygiene procedures (*i.e.* tooth brushing, use of dental floss). In general, there are no age or sex differences with respect to the type and frequency of professional dental care received by, or the oral hygiene habits of, study participants. Thus, this is a reasonably homogenous population, quite useful to describe oral physiological status across the human life span.

Several general dental characteristics were evaluated. The number of decayed, missing or filled teeth (DMFT) increased with age among men, and with post-menopausal status among women. The total number of natural teeth present in subjects decreased with age [∿26 for young men (20-39 Y), ∿25 for middle-age men (40-59 Y), ∿20 for older men (≥60 Y); ∿26 for pre-menopausal women, ∿23 for post-menopausal women]. It is interesting, however, that among the older persons in this study, most are substantially dentate. An increased prevalence of root surface caries (cervical caries) has been previously noted in older populations. A strong correlation between age (or post-menopausal status, for women) and prevalence of cervical caries (total-active and filled) was noted in this study; for men - 1.03, young; 2.21, mid-age; 4.2, old; for women - 0.8, pre-menopausal; 4.1, post-menopausal. Gingival health was evaluated and no differences were observed between any age or sex grouping. There was a strong relationship noted between age (or post-menopausal status) and the periodontal disease index. Older individuals showed significantly greater levels of periodontal diseases than their younger counterparts.

Exocrine protein secretion was evaluated following 2%-citrate stimulation of salivary flow from the parotid gland. The release of the acidic proline-rich proteins (PRP) into saliva was used as an index of this process. The PRP have previously been shown to be located within secretory granules of human parotid acinar cells and thus are an appropriate marker for parotid protein exocytosis. Data from 220 participants were utilized. Measurement of PRP was accomplished by a micro-immunodiffusion assay, with antisera specifically directed at the human PRP. No alterations, for both men and women, in the concentration of PRP in stimulated parotid saliva were observed across different age groups. Average values in mg% (± SEM) were: for men - 49.7 young, 46.7 mid-age, 41.7 old; for women - 52.1 young, 50.9 mid-age, 45.7 old. Also the percentage of total protein, represented by the PRP (%PRP), remained constant with age (total protein content in saliva was also stable with age). Observed values for % PRP for men were 10.9%, young, 17.9%, mid-age, 17.0%, old; for women - 19.1%, young, 16.4% mid-age, 15.2%, old. These data suggested that exocrine protein secretion remains unaltered with age. Since little data is available generally on exocrine secretory processes with age, as well as specifically with salivary glands, this finding requires further study. One approach, which we are now utilizing, is to evaluate other known exocrine proteins of the parotid gland (*i.e.* proteins shown to be present in secretory granules of acinar cells) to see if the initial conclusion reached with the PRP are generally applicable or restricted to that group of secretory molecules.

Data from over 120 subjects, on determined taste thresholds for each of the 4 basic taste modalities (sweet, sour, salty and bitter), have been analyzed. Results with sodium chloride demonstrate a modest, but significant decrease in the ability of older persons (men and women) to detect threshold levels of this tastant (*i.e.* the threshold increases with age, $r=0.38$, F ratio=14.9, $p<.0005$). This change is gradual, occurring throughout the lifespan, rather than being a precipitous alteration only in the elderly. For quinine sulfate, a bitter taste, a lesser but also significant diminution in detection threshold ability occurs with increased age ($r=0.25$, F ratio=5.3, $p<.025$). The nature of this alteration is best described as gradual, like that with sodium chloride. The taste detection thresholds for both sweet (sucrose) and sour (citric acid) modalities are not significantly affected by age. However, a sex difference in the threshold for citric acid was observed (F ratio=6.6, $p<.012$).

With completion of this phase in analysis of data on taste detection thresholds, we are beginning to evaluate results of parallel studies with the same individuals on perception of taste intensities. These two tests measure distinctly different processes: thresholds describing the lowest concentration of a tastant which can stimulate a tastebud, and perception of tastant intensities describing an individuals ability to scale taste using real world, suprathreshold concentrations of tastants. Preliminary findings of the latter studies, analagous to those of threshold determinations, suggest quality specific variations apparent with different aged persons. In particular, significant differences seem to be present among older men in the intensity with which they perceive suprathreshold levels of sodium chloride and, among older women, differences are observed in intensity perception of sweet tastes. In both of these cases, intensity perceptions of suprathreshold tastant concentrations, are reduced in older persons.

Significance to Biomedical Research and to the Program of the Institute: This study has begun to define the physiologic status of the oral cavity with increased age. As witnessed by our results, our approach apparently allows for the distinction between age-associated oral physiology and alterations related to disease or therapeutic treatment. Thus, a re-examination of many "traditional" stereotypes, associated with old age and oral health, is possible. Any alterations in salivary gland functions, because of the critical role for saliva in oral health maintenance, may have broad oral effects. Changes in the dental apparatus can have markedly adverse effects on facial appearance, speech and dietary patterns. Changes in oral motor and gustatory functions can affect the quality of one's life. By providing basic descriptions of oral physiological functions with age, this study will significantly contribute to a rational assessment of what impact changes in oral function may have on the aging individual. In addition, such information should be quite useful for oral health care planning by helping to define the oral health needs of the aged.

Proposed Course of the Project: Examinations of oral tissues, as described above, will continue. Substantial effort will be placed on collating already obtained results, analyzing their meaning, and drawing appropriate conclusions.

Publications:

Baum, B. J.: Current research on aging and oral health. Special Care in Dentistry 1: 105-109, 1981.

Cole, M. F., Hsu, S. D., Baum, B. J., Bowen, W. F., Sierra, L. I., Aquirre, M., and Gillespie, G.: Specific and nonspecific immune factors in dental plaque fluid and saliva from young and old populations. Infect. Immun. 31: 998-1002, 1981.

Baum, B. J.: Evaluation of stimulated parotid saliva flow rate in different age groups. J. Dent. Res., in press.

Baum, B. J.: Characteristics of participants in the oral physiology component of the Baltimore Longitudinal Study of Aging. Community Dent. Oral Epidemiol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00050-03 LMA

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of Salivary Gland Function During Increased Age.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. J. Baum	Dental Officer	LMA, NIA
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COOPERATING UNITS (if any)

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LAB/BRANCH
Gerontology Research Center, Laboratory of Molecular Aging

SECTION
Intermediary Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.8	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous clinical observations have suggested that with increased age, decremental changes in salivary gland function occur. To study the mechanism of this alteration in animal models, salivary glands from young adult and aged male and female rats have been characterized biochemically and their regulation by neurotransmitters has been investigated. Included are studies of: (1) protein secretion from the parotid gland and its relationship to β-adrenergic stimulated endogenous protein phosphorylation; and (2) fluid and electrolyte section (K^+ efflux, Ca^{2+} efflux, α-adrenergic and cholinergic responsiveness) from the parotid gland. In the submandibular gland, basal protein biosynthesis and release has been characterized. The role of both α- and β-adrenergic receptor stimulation in maximum exocrine protein secretion from the submandibular gland has been evaluated.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: These studies have as their overall objective to understand the biochemical mechanisms of saliva formation (*i.e.* regulation of the synthesis of secretory macromolecuels and control of the release of salivary proteins and electrolytes into saliva) and the alterations occurring in these processes during aging. Constituents of saliva (organic, inorganic) have direct maintenance and protective functions in the oral cavity. Age-related perturbations in the formation and secretion of salivary gland products would likely be manifested as compromises to oral health.

Methods Employed: Rat salivary glands were used as model tissues. Descriptive biochemical characterizations of glands included information of DNA, protein, neutral sugar and sialic acid contents. In addition, the contents of several specific proteins were determined by immunological (ELISA) or enzymological assays. *In vitro* studies were performed with enzymatically dispersed cells from parotid and submandibular glands. α-Adrenergic receptors were measured using either [^{3}H]dihydroergocryptine or [^{3}H]prazosin. Adrenergic and cholinergic receptor mediated events were studied using appropriate agonists and antagonists. Protein secretion was monitored by release of amylase or previously radiolabeled exocrine proteins. Fluid and electrolyte release was studied as K^+ and $^{45}Ca^{2+}$ efflux. Membrane phospholipid turnover was measured as change in the specific radioactivity of [^{32}P]-Pi labeled phosphatidic acid and phosphotidylinositol. Protein biosynthesis was studied using pulse-chase methodology with appropriate radiolabeled amino acid precursors. Newly synthesized macromolecules were characterized by gel electrophoresis and autofluorography. Cyclic AMP stimulated events, subsequent to β-adrenergic agonist binding, were followed by protein kinase activity ratios and endogenous phosphorylation of salivary gland proteins. Newly phosphorylated proteins were identified by SDS gel electrophoresis, autoradiography, gel slicing and densitometry. *In vivo* studies were performed with anesthetized rats. Ducts from the parotid and submandibular glands were cannulated intraorally and gland saliva was collected following stimulation with appropriate agonists.

Major Findings: Dispersed submandibular gland cells were utilized to evaluate new protein production and release. In these cells, as opposed to acinar cell preparations from the parotid gland, both α- and β-adrenergic receptor stimulation were required for maximum exocrine protein release. Both separate adrenergic agonist and antagonist experiments support this conclusion. A broad size range of newly synthesized proteins were detected in soluble cell and media fractions from cell incubations ($M_r \sim 10^4 - 5 \times 10^5$). Adrenergic stimulation (l-epinephrine) specifically increased the secretion of certain radiolabeled proteins, and, in addition resulted in both cellular and secreted proteins with electrophoretic characteristics distinct from that of control preparations. Thus, adrenergic stimulation of these cells not only promoted secretion but profoundly affected protein processing as well.

β-Adrenergic stimulation of dispersed parotid gland cells stimulated a rapid elevation of *in situ* cAMP-dependent protein kinase activity (4 fold, within 30 sec). The secretion of amylase from these cells was delayed until 1-2.5 min after neurotransmitter addition. β-Adrenergic stimulation also resulted in 3 specific changes in parotid phosphoprotein phosphate content (followed as ^{32}P).

These included 2 increases in ^{32}P content (M_r ∿27,000 and 14,000) and 1 decrease in ^{32}P content (M_r ∿13,600). All three changes were quite rapid (within 30 sec) and were mimicked by addition of dibutyryl cAMP in place of the β-adrenergic agonist. Changes in ^{32}P content of the 3 proteins was dependent on the concentration of agonist present. All showed half-maximal responses with ∿10^{-8} M l-isoproterenol. The dose dependent showed good agreement with that of cAMP-dependent protein kinase activation and amylase release. The results suggest a possible role for these 3 parotid phosphoproteins in protein exocytosis.

Several descriptive characteristics of parotid glands from young adult (6 mo) and aged (23, 24 mo) male rats were determined. No differences in gland DNA content were found. However, a significant decrease in total protein content was observed (young = 17.3 mg/mg DNA, old = 14.3 mg/mg DNA). Also, the gland content of amylase was decreased approximately in half. Amylase activity in young animals = 3500 U/mg DNA and in old animals was 1876 U/mg DNA. Immunoreactive amylase from young animals was 11.4 mg/mg DNA while that in old animals = 6.1 mg/mg DNA. This suggests an alteration in secretory protein production (synthesis, degradation) in parotid glands from different aged animals.

α-Adrenergic receptors in rat parotid glands were characterized. With ^3H dehydroergocryptine as a radioligand, a heterogeneity in receptor sub-type could be discerned by studying displacement of ligand with prazosin (relatively specific for α_1 sub-types) and yohimbine (relatively specific for α_2 sub-types). Using prazosin, 2 distinct populations of receptors were seen, in about equal proportions. The binding of [^3H]prazosin to parotid membranes was rapid, reversible, saturable and stereospecific, and showed a high affinity (K_D = 0.38 nM) and low capacity (V_{max} = 380 fmol/gm tissue). The characteristics of [^3H]prazosin binding were in good agreement with results from catecholamine stimulated K^+ release. This suggests that K^+ release from the rat parotid gland is an α_1-adrenergic mediated event.

Earlier findings from this laboratory noted marked response differences in K^+ efflux following α-adrenergic stimulation of parotid gland cells from young and aged rats. When cells from young animals were pre-incubated with $^{45}Ca^{2+}$ and then exposed to 10^{-5} M l-epinephrine, a rapid rate of Ca^{2+} efflux was noted (∿6.0%/min). Similar studies with cells from aged rats showed a significant diminution (approximately 70% lower) in mobilization of cellular $^{45}Ca^{2+}$ pools. Such alterations in α-adrenergic responsivity were not due to changes in α-adrenergic receptor characteristics. Both the receptor number (19.8 fmol/mg protein, 3 mos; 32.7 fmol/mg protein, 12 mos; 33.3 fmol/mg protein, 24 mos) and affinity (K_D) (0.65 nM, 3 mos; 1.1 nM, 12 mos; 1.35 nM, 24 mos) did not change in a manner consistent with a receptor defect. The evaluation of α-adrenergic mediation of phosphatidylinositol (PI) turnover did however show an age-related decrement, consistent with alterations in K^+ and Ca^{2+} efflux. PI turnover in 24 mos rats was reduced about 45% ($p<0.01$) from that seen in young adult rats.

Significance to Bio-medical Research and the Program of the Institute: These studies continue the examination of salivary gland function throughout the adult life span of the rat. Based on morphologic and biochemical data, the rat salivary glands are suitable models for the human condition. Many clinical studies have suggested a variety of age-related changes in the human oral cavity likely, at least in part, to be related to decrements in salivary gland function. These include complaints of xerostomia, increased prevalence of cervical caries, alterations in taste acuity and perception, "atrophy" of the oral mucosa. Salivary constituents include a number of factors which function in the maintenance of oral health. By studying the mechanisms which regulate salivary gland function, one can begin to understand physiological malfunctions and thus be better equipped to design therapeutic regimens.

Proposed Course of the Project: Further studies with dispersed submandibular gland cells will focus on the mechanisms involved in regulation of new exocrine protein synthesis by adrenergic agonists. This *in vitro* model will also be utilized with aged rat tissue. Relative rates of protein production, and electrophoretic characteristics of newly synthesized proteins, will be studied with different aged rats. Hormone responsiveness in tissue from senescent rats will be compared to that from young adult animals.

The observed age-related decrements in α-adrenergic mediated parotid gland functions will be further studied. This includes more detailed examination of the role of Ca^{2+} in these α-adrenergic responses. Furthermore, cholinergic responses in various aged animals will be studied. α-Adrenergic and cholinergic responses are thought to share several common regulatory mechanisms. Thus it will be important to determine if both types of responses are similarly affected. Production of exocrine protein will be studied in dispersed parotid cells. Methods developed with the submandibular gland will be used to examine rates of synthesis, turnover and macromolecule characteristics, with young adult and aged rat parotid cells. Also, immunological methods will be developed to assess rates of production with specific proteins.

The *in vivo* model will be used to (1) describe stimulated gland salivas from young and old rats; (2) to evaluate the secretion of newly synthesized proteins into gland saliva after *in vivo* radiolabeling procedures.

Publications:

Baum, B. J., Colpo, F. T. and Filburn, C. R.: Rat parotid gland cyclic AMP-dependent protein kinase: characterization and relationship to exocrine secretion. Arch. Oral Biol. 26: 333-337, 1981.

Ito, H., Baum, B. J. and Roth, G. S.: β-Adrenergic regulation of rat parotid gland exocrine protein secretion during aging. Mech. Ageing Dev. 15: 177-188, 1981.

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Kuyatt, B. and Baum, B. J.: Characteristics of submandibular glands from young adult and aged rats. J. Dent. Res. 60: 936-941, 1981.

Baum, B. J. and Kuyatt, B. L.: Protein production and release by dispersed rat submandibular gland cells *in vitro* after adrenergic stimulation. Life Sci., in press.

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Bienkowski, R. S. and Baum, B. J.: Measurement of intracellular protein degradation. In Adelman, R. and Roth, G. (Eds): Methods in Aging Research. Detection of Altered Proteins. Boca Raton, CRC Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00051-01 LMA

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Phosphate and Calcium Homeostasis, Relation to Senile Osteoporosis and Osteomalacia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Intermediary Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.4	6.4	3.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Osteoporosis is a leading cause of mortality and morbidity in aging women. The mechanisms of phosphate and calcium balance were studied as part of a major effort to understand the pathophysiological bases of senile osteoporosis and osteomalacia. This project represents a continuation and extension of previous projects - Z01 AG 00041-07 LMA and Z01 AG 00042-07 LMA.

Notable scientific achievements include: (1) The active form of Vitamin D, 1,25-dihydroxy Vitamin D_3, was found to enhance specifically the sodium gradient-dependent phosphate uptake system in the kidney; (2) Vitamin D was found to have a direct effect on renal membrane phospholipids, resulting in an altered permeability towards calcium; (3) Evidence for a novel parathyroid hormone-independent mechanism for regulating phosphate reabsorption by the kidney was obtained; and (4) The mechanism by which calcium is actively transported in the kidney was described.

Project Description:

Objectives: Osteoporosis is a leading cause of mortality and morbidity in aging women. A study of the mechanisms of phosphate and calcium balance was initiated as part of a major effort to understand the pathophysiological bases of senile osteoporosis and osteomalacia. This investigation was prompted by our pioneering development of technology for the isolation of specific plasma membrane vesicles, the use of these isolated membrane vesicles to define transport mechanisms and by the findings that hormones, *i.e.* parathyroid hormone, calcitonin, and perhaps estrogen, prolactin and insulin; 1,25-dihydroxy vitamin D_3; diet; and chronic uremic syndromes, induce specific alterations in the activities of the membrane transporters that are reflected in changes in the absorption of phosphorus and calcium by the kidney and intestine.

Methods Employed: Model systems representing various levels of organization were used. These range from membrane vesicles derived from the luminal (brush border) and basal-lateral segments of the plasma membranes of renal and intestinal cells; intact cells, isolated or grown in culture; to the intact animal.

Major Findings:

The possible clinical use of Vitamin D_3 and its metabolites in postmenopausal osteoporotic women tends to obscure the fact that little is known about the function of Vitamin D and in particular about its biochemical mechanism of action. To provide this needed information, last year we established an experimental animal facility to produce Vitamin D-deficient chicks to be used as a model. The effect of Vitamin D status on the uptake of phosphate by kidney cells was examined. A new preparation of cells, isolated by a hyaluronidase-collagenase method, was developed and the cells were shown to be viable and metabolically active. The isolated cells accumulated phosphate and α-methyl-D-glucoside (a non-metabolizable sugar analog) in response to a Na^+ gradient. It was found for the first time that *in vivo* administration *i.o.* of 1 µg of 1,25-dihydroxy Vitamin D_3 to rachitic chicks effected within 3 hrs a 60% increase in the Na^+ gradient-dependent uptake of phosphate in the isolated cells. The specificity of the response was evident from the findings that the vitamin did not alter the uptake of phosphate in the absence of a Na^+ gradient, nor the uptake of the sugar in the presence or absence of the Na^+ gradient. This initial response to Vitamin D_3 administration to the animal also brought about a secondary alteration, an increase in the serum level of phosphate. With the resultant hyperphosphatemia, Na^+-dependent phosphate uptake by cells isolated 1 day after injection of the vitamin was inhibited.

The pioneering observation was made that 1,25-dihydroxy Vitamin D_3 incubated with kidney cells from D-deficient animals *in vitro* caused an increase in the Na^+-dependent phosphate uptake. The effect was seen within 1 hr and was concentration-dependent. A concentration as low as 10^{-14} M produced stimulation. Estimates from the uptake of the labeled vitamin indicated that, on the average, only 250 molecules of 1,25-dihydroxy Vitamin D_3 per cell was needed to stimulate uptake, and probably this value represented a considerable over-estimation. Cells from Vitamin D_3-repleted chicks did not respond to the vitamin when incubated *in vitro*.

The Vitamin D status of the animal was also reflected in the intestinal uptake of phosphate and calcium. For phosphate, cells isolated from the jejunum showed enhanced uptake (2-fold) after the *in vivo* administration of 1,25-dihydroxy Vitamin D_3. For calcium, cells of the ilium were most responsive.

It has been generally held that Vitamin D, like other steroid hormones, brings about its physiological effect by an interaction of the vitamin with intracellular receptors, transfer to the nucleus, and transcription of specific genes which code for transport proteins. Important initial experiments in our Laboratory now suggest an alternative mechanism, *i.e.* a direct effect of the vitamin on the membrane phospholipids, resulting in an altered permeability towards phosphate and calcium.

Another important determinant to the understanding of the role of Vitamin D in phosphate and calcium homeostasis is the regulation of the synthesis of the active form of the vitamin, 1,25-dihydroxy Vitamin D_3. Previous studies showed that the enzyme 1-hydroxylase which catalyzes the synthesis of the active form of the vitamin is localized in kidney mitochondria and its activity is controlled by various factors including serum levels of calcium, phosphate and parathyroid hormone as well as substrate, 25-hydroxy Vitamin D_3, and product, 1,25-dihydroxy Vitamin D_3. The mechanisms of regulation are unknown. Using isolated renal cells of D-deficient chicks and mitochondria derived from the cells it was found that the 1-hydroxylase was inhibited by 1,25-dihydroxy Vitamin D_3, *i.e.* the active form of the Vitamin D_3 regulated its own synthesis.

Studies were initiated on the mechanism by which the level of phosphate in the serum regulates renal phosphate transport. In the thyroparathyroidectomized rat, the acute infusion of phosphate increased the net tubular reabsorption of phosphate for 60 min but, thereafter, tubular reabsorption decreased. Glomerular filtration rate and urine flow were constant throughout. Importantly, this adaptation was reflected at the level of the brush border membrane. Brush border membrane vesicles, isolated from rats at the start of the infusion, accumulated phosphate at a rate of 1500 pmol/5 s•mg protein whereas uptake decreased to 880 pmol/5 s•mg protein after infusion. This parathyroid hormone-independent mechanism of regulating phosphate reabsorption by the kidney has great physiological and clinical significance and is being actively investigated.

A project was initated to examine the mechanism by which calcium is transported transepithelially across the renal tubules. Thermodynamic considerations would suggest that the transport of Ca^{2+} from lumen to cell across the brush border membrane is down an electrochemical gradient and, therefore, mediated by a facilitated diffusion system. In contrast, movement of Ca^{2+} from cell to blood across the baso-lateral membrane is against its electrochemical gradient and, therefore, should require an active transport process. Using isolated baso-lateral membrane vesicles, prepared by a new technique (described below), a Ca^{2+}-Na^+ antiport system was identified which would enable Ca^{2+} to be pumped out of the cell in exchange for Na^+ entering down its electrochemical gradient. The effect of imposed membrane potentials indicated that the system was electrogenic, *i.e.* more than 2 Na^+ were exchanged for each Ca^{2+}. The apparent Km values were 66 μM for Ca^{2+} and 15 mM for Na^+. The flux through the system was sufficiently great to suggest that this exchange reaction could represent the major pathway and driving force for energizing the reabsorption of Ca^{2+} in the kidney.

Significance to Biomedical Research and to the Program of the Institute: These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: To investigate the pathophysiological basis of senile osteoporosis and osteomalacia.

Publications:

Cheng, L. and Sacktor, B.: Sodium gradient-dependent phosphate transport in renal brush border membrane vesicles. *J. Biol. Chem.* 256: 1556-1564, 1981.

Liang, C. T., Takenawa, T. and Sacktor, B.: Regulation of hormone (PTH and PGE_1)-stimulated adenylate cyclase by renal cytosolic factors. *Mol. Cell. Endocrinol.* 21: 221-231, 1981.

Cheng, L., Liang, C. T. and Sacktor, B.: *In vitro* effects of parathyroid hormone on kidney cortical slices: cAMP responses and concomitant inhibition of the Na^+ gradient-dependent uptake of phosphate by brush border membrane vesicles isolated from the renal slices. *Endocr. Res. Commun.*, in press.

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Sacktor, B., Cheng, L. and Liang, C. T.: *In vivo* and *in vitro* effects of vitamin D_3 [$1,25(OH)_2$-D_3] on the uptake of phosphate (Pi) by chick kidney cells. *Proceedings of the 5th International Workshop on Phosphate and Other Minerals*, New York, September 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00052-01 LMA

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pathophysiological and Hormonal Regulation of Membrane Transport Systems.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	D. Sogin	Expert (DOD 7/81)	LMA, NIA
	U. Reiss	Visiting Associate	LMA, NIA
	T. Uchida	Visiting Fellow	LMA, NIA
	D. Spector	Department of Medicine	Johns Hopkins U.
	E. Krauss	Department of Medicine	Johns Hopkins U.
	G. Briefel	Department of Medicine	Johns Hopkins U.

COOPERATING UNITS (if any)

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SECTION

Intermediary Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.7	6.2	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project studies the pathophysiological and hormonal regulation of membrane transport systems and the mechanisms by which age-dependent changes perturb physiological control systems and, thus, contribute to the failure to maintain homeostasis in the aged. This project represents a continuation and extension of previous projects - Z01 AG 00041-07 LMA and Z01 AG 00042-07 LMA. Notable scientific achievements include: (1) The discovery was made that glucocorticoids increase sodium-proton exchange activity which represents the major mechanism for acid secretion and bicarbonate reabsorption in the proximal tubule; (2) In chronic uremia, sodium gradient-dependent phosphate uptake was decreased. Thyroparathyroidectomy restored the activity; (3) Parathyroid hormone-mediated decrease in phosphate uptake was found to be concomitant with the phosphorylation of specific membrane proteins; (4) Membrane enzymes from aged animals was discovered to have altered conformation with increased antigenic sites; (5) Hybridoma technology for the production of monoclonal antibodies against specific membrane carriers was developed to determine how the proteins change during aging and in different pathophysiological states; (6) A new technique for the preparation of renal cortical baso-lateral membrane vesicles was developed.

Project Description:

Objectives: These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in regulation of transport processes perturb physiological control systems and, thus, contribute to the failure to maintain homeostasis in the aged. This new information is needed as a base for the eventual development of appropriate techniques and procedures that will prevent and/or enable the aged to cope effectively with their debilities.

Methods Employed: Appropriate biochemical, physiological and endocrinological techniques for specific experimental questions are developed and/or adapted, as reported in published papers.

Major Findings:

A novel discovery, that glucocorticoids regulated specific transport systems in the kidney, was made. Previous studies had speculated that glucocorticoids could alter proximal tubular function for it was noted that glucocorticoids changed the acid-base status and net acid excretion of man and animals and also caused a parathyroid hormone-independent phosphaturia. These reports become explicable with our finding that glucocorticoids *in vivo* enhanced Na$^+$-H$^+$ exchange in brush border membrane vesicles. The treatment groups were: I. sham operated; II. sham + high dexamethasone; III. adrenalectomy (ADX); IV. ADX + aldosterone; and V. ADX + maintenance dexamethasone. The activity of the Na$^+$-H$^+$ carrier, measured by the uptake of ^{22}Na$^+$ in the presence of a pH gradient (pH$_i$ = 5.5, pH$_o$ = 7.5), in groups I, III and IV were the same. Transport in group II was 70% greater than that in group I. Transport in group V was 50% greater than that in group III. These results indicated that glucocorticoids, but not mineralocorticoids, increased exchange activity. The Na$^+$-H$^+$ exchange represents the major mechanism for acid secretion and bicarbonate reabsorption in the proximal tubule. The antiport system also represents a major pathway for Na$^+$ reabsorption, independent of the co-transport systems. Thus, the glucocorticoid-enhanced exchange activity should be concomitant with a dissipation of the Na$^+$ gradient driving force, resulting in a decrease in the activity of Na$^+$-cotransport systems in the presence of a Na$^+$ gradient but not in the absence of the gradient. This was precisely found. Na$^+$ gradient-dependent phosphate and D-glucose uptakes were inhibited in groups II and V. In the absence of the gradient, *i.e.* Na^+_i = Na^+_o, uptakes in all groups were the same. Thus, the previously observed phosphaturia is now explained.

It was known that in chronic uremia or in pathophysiological states in which nephrons are lost, such as occurs in aging, compensatory mechanisms are invoked to maintain phosphate homeostasis. The nature of these mechanisms is unknown and it represents one of the most active areas of investigation by renal and endocrine physiologists. Making use of the expertise inherent in the Laboratory of Molecular Aging, we recently initiated a project in collaboration with investigators at Baltimore City Hospitals and The Johns Hopkins School of Medicine to explore these compensatory mechanisms. The experimental animal model was the rat made uremic (BUN increased 4-times) by the removal of one kidney and the ablation of two-thirds of the remaining kidney. In important, but preliminary findings, we found that Na$^+$ gradient-dependent phosphate transport was decreased. Thyroparathyroidectomy restored the activity. The uremic animal, however, still responded to a low phosphorus diet by increasing phosphate transport. Na$^+$-H$^+$ exchange was not affected.

Parathyroid hormone increases phosphate excretion by inhibiting phosphate transport in the renal tubule. Although the mechanism by which the hormone inhibits transport is unknown, it was found that the action of the hormone, *in vivo*, was reflected in an alteration of the Na^+-phosphate co-transport system in the isolated brush border membrane of the proximal tubular cell. Further studies, *in vitro*, demonstrated that membrane vesicles, isolated from renal cortical slices pre-incubated with ^{32}P to label ATP, and then incubated with either parathyroid hormone or dibutyryl cAMP, had an altered uptake of phosphate and a modified state of protein phosphorylation. Na^+ gradient-dependent phosphate uptake was inhibited. The phosphorylation of 5 proteins having molecular weights of 170 K, 135 K, 105 K, 88 K, and 68 K were enhanced by the hormone or the cAMP analog. Phosphorylation of isolated membrane vesicles before and after disruption with detergent indicated whether the proteins faced the luminal or the cytosolic side of the membrane. Ca^{2+} also affected the phosphorylation of specific proteins.

Last year, we reported the establishment of a tissue culture facility and the use of cultured kidney cells to study transport mechanisms and their regulation. Na^+ gradient-dependent phosphate and sugar systems were characterized. The uptake of phosphate was found to be inhibited by parathyroid hormone and vasopressin, hormones which in these cells induced the synthesis of cAMP and increased protein kinase activity, but uptake was not appreciably affected by glucagon nor isoproterenol, hormones which in these cells did not significantly affect cyclic nucleotide metabolism. Initial studies indicated that Forscolin, a newly described potent activator of adenylate cyclase, inhibited phosphate transport and increased protein kinase activity markedly.

The uptake of D-glucose by renal brush border membrane vesicles was studied in the absence of Na^+. Uptake of the sugar was membrane potential (inside negative)-dependent, inhibited by phlorizin, stereo- and sugar-specific, accelerated by exchange diffusion, saturable, and temperature-dependent. The binding of phlorizin, in the absence of Na^+, was also increased by a membrane potential (inside negative). Thus, the properties of this membrane potential-dependent, Na^+-independent sugar transport system resembled those described for the Na^+-D-glucose co-transport system. In the absence of Na^+, but presence of a valinomycin-induced K^+ diffusion potential, the apparent Km for D-glucose was 43 mM. This contrasted with an apparent Km of 1.8 mM for the Na^+ chemical gradient system. Therefore, the Na^+-independent uptake system represented a low affinity transport mechanism. It is suggested that the same carrier mediates the Na^+-independent and Na^+-dependent transport systems. Hypothetical models for the membrane potential-dependent stimulation of D-glucose uptake, in the absence of Na^+, have been proposed.

Last year, we reported on the age-dependent changes in the molecular structures and activity of the renal brush border membrane enzyme, maltase. In continuing studies we found that the enzyme from old (24 mo) had more (1.5 to 3.0-fold) antigenic sites per mg protein than the enzyme from mature (6 mo) animals. These results, together with earlier findings of the changes in enzyme conformation as a function of age, prompted us to try to develop monoclonal antibodies specific for the old and/or young molecules. Preliminary results were encouraging.

The technology of the production of monoclonal antibodies was adapted and was used as a means to resolve the difficult problem of the purification and characterization of specific renal brush border membrane carriers and how they might be changed during aging and in different pathophysiological states. Using other techniques, previous studies from several laboratories were not very successful due to the insolubility of the transport proteins in aqueous solution, their low frequency in the membrane, and the lack of an adequate assay for activity as the proteins were purified. In current studies, several cultures of fused spleen-myeloma cells produced antibodies that inhibited specific transport systems and, thus, proved to be strong candidates for cloning. Thus, promising leads were developed which need to be exploited.

A membrane preparation enriched in the baso-lateral segment of the plasma membrane was isolated from the rat renal cortex by a procedure that included separation of particulates on a self-generating Percoll gradient. The uptake of L-glutamate by the baso-lateral membrane vesicles was studied. A Na^+ gradient ($[Na^+]_o > [Na^+]_i$) stimulated the uptake of L-glutamate and provided the driving force for the uphill transport of the acidic amino acid, suggesting a Na^+ L-glutamate co-transport system in the baso-lateral membrane. A K^+ gradient ($[K^+]_i > [K^+]_o$) increased the uptake additionally. This effect was specific for K^+ (Rb^+). The action of the K^+ gradient in enhancing the uptake of L-glutamate had an absolute requirement for Na^+. In the presence of Na^+, but in the absence of a Na^+ gradient, *i.e.* $[Na^+]_o = [Na^+]_i$, the K^+ gradient also energized the concentrative uptake of L-glutamate. This effect of the K^+ gradient was not attributable to an alteration in membrane potential. The finding of a concentrative uptake system for L-glutamate energized by both Na^+ ($[Na^+]_o > [Na^+]_i$) and K^+ ($[K^+]_i > [K^+]_o$) gradients in the baso-lateral membrane, combined with previous reports of an ion gradient-dependent uphill transport system for this amino acid in the brush border membrane, suggests a mechanism by which L-glutamate is accumulated intracellularly in the renal proximal tubule to extraordinarily high concentrations.

The regulation of phosphatidylinositol turnover by α-adrenergic agonists in rat parotid acinar cell aggregates was examined with respect to kinetics and agonist-antagonist interactions. Phosphatidylinositol turnover was followed by the changes in the specific activities of ^{32}P-phosphatidic acid and ^{32}P-phosphatidylinositol. The specific activity of phosphatidic acid increased rapidly (within 1 min) after addition of epinephrine (10^{-5} M), reached a maximum level by 12 to 16 min, and then decreased. Incorporation of ^{32}P into phosphatidylinositol exhibited a lag phase of about 5 min and then increased continuously for an additional 40 min. The absolute amounts of phosphatidic acid and phosphatidylinositol did not change. The concentrations of epinephrine needed to stimulate ^{32}P incorporation into phosphatidic acid and phosphatidylinositol, measured at 15 and 30 min, respectively, were similar; K_a values of $2.05 \pm 0.46 \times 10^{-6}$ M for phosphatidic acid and $2.98 \pm 0.30 \times 10^{-6}$ M for phosphatidylinositol were found. The effects of agonists on ^{32}P labeling of phosphatidylinositol were, in order of potency, epinephrine$\geq$norepinephrine>phenylephrine>>normetanephrine. When various adrenergic antagonists were evaluated for their ability to inhibit 10^{-5} M epinephrine-stimulated ^{32}P incorporation into both phosphatidic acid and phosphatidylinositol, the order of antagonist potency was prazosin$\geq$phenoxybenzamine>phentolamine>yohimbine>>>propranolol. These findings indicate that phosphatidylinositol-phosphatidic acid turnover in the rat parotid gland is mediated by the α_1-adrenergic receptor system.

Significance to Biomedical Research and to the Program of the Institute: These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of Study: The pathophysiological and hormonal regulation of membrane transport systems will be examined and the mechanisms by which age-dependent changes perturb physiological control systems will be studied.

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Annual Report of the Laboratory of Neurosciences
National Institute on Aging

The Laboratory of Neurosciences at the National Institute on Aging was formed in 1978, and has embarked on a program of research on the central and peripheral nervous systems and muscle, in health, disease and aging. This report summarizes the following projects: (A) Synapse development, specificity and mechanism in culture, (B) Blood-brain barrier and central nervous system function, (C) Transport systems at the blood-brain barrier, (D) Function of peripheral nerve and muscle, (E) Drug pharmacokinetics, relation to pharmacodynamics and senescence, (F) Central nervous system binding sites for the neurotoxin kainic acid, (G) Pharmacology of central and peripheral catecholaminergic systems, (H) Assessment of neurochemical markers in relation to age and behavior, (I) Cerebral metabolism, relation to brain function and aging, and (J) Brain function in aging and disease in man. Dr. J.M. Thompson is primarily responsible for project _A_, I am responsible for project _B_, Dr. Q.R. Smith for project _C_, Drs. H. Levitan, N. Shinowara, M. Michel and P. Ask for project _D_, Dr. I. Kapetanovic for project _E_, Dr. E. London for project _F_, Dr. M. Partanen for project _G_, Dr. S. Waller and E. London for project _H_, Dr. E. London for project _I_, and Drs. R. Margolin and R. Duara for project _J_.

A. Synapse Development, Specificity and Mechanism in Culture.

Dr. J. Thompson demonstrated that synapse formation, synapse termination, synapse specificity and neurotransmission can be studied using neurons and muscle cells in tissue culture. He detected functional synapses by inserting microelectrodes into muscle cells, and recorded miniature end plate potentials in these cells. He demonstrated that neurons from retina or chick spinal cord form long-lived synapses with muscle cells in culture. Each type of neuron, therefore, possesses a synapse-competent state during its development, when it can form abundant synapses with muscle cells in culture. Dr. Thompson's results suggest a mechanism of synapse specificity that is based on stabilization of correct synapses which is coupled with a synaptogenic period during development for each type of neuron. Dr. Thompson's studies also demonstrate that synapse formation and retention can be studied in a controlled fashion in tissue culture. His findings may be critical for our interpretation of neuronal cell death in the aging nervous system.

Specifically, Dr. Thompson showed that rat retinal neurons formed synapses only between the 18th day of embryonic development and day 7 after birth, with a maximum number formed from pups 1 day old. Neurons from the chick embryo spinal cord formed synapses after 2 days, the maximum number with 4-day neurons. The capacity to form synapses decreased with age, and was observed after 51 hr but not after 24 hr of co-culture with neurons from 18-day embryo spinal cord. Thus, spinal cord neurons do not completely lose their ability to form synapses with muscle cells. All neurons tested show decreased ability to form synapses as they aged during development. The neurons also lose their ability to form synapses when maturing _in vitro_ in a manner and time course which parallels the loss of their ability to form synapses during _in ovo_ maturation. Neurite extension by neurons _in vitro_ also changed with development. There was a statistically significant age-related decrease in the percentage and average length of neurites formed in 24-hr cultures of chick retina and spinal cord neurons between 6- and 16-days of embryonic age. These results imply that maximal rates of neurite extension and synaptogenesis occur concurrently during development and suggest that neurite extension is critical to synaptogensis.

In tissue culture studies, neuroblastoma cells lines have become important *in vitro* models for neuronal systems. Dr. Thompson demonstrated, contrary to current opinion, that three commercial neuroblastoma lines are not homogeneous, and that more than one cell type is presented in them. This means that studies with them may have to be re-evaluated.

B. Blood-Brain Barrier and Central Nervous System Function.

Studies of the blood-brain barrier in health, disease and aging require quantitative measurements of cerebrovascular permeability and transport, and adequate pharmacokinetic theories to interpret drug distribution within the various brain compartments in relation to plasma concentrations and metabolism. Current techniques for measuring cerebrovascular permeability and transport are insensitive, semi-quantitative and can be influenced by changes in cerebral blood flow. My colleagues and I therefore elaborated and tested a new quantitative procedure to measure blood-brain barrier permeability that can be used in conscious animals independent of cerebral blood flow and is at least 1000 times more sensitive than any other currently available technique.

A major problem in clinical psychopharmacology is that a central drug response often cannot be related directly to drug dosage or plasma concentration. In the elderly, furthermore, the high incidence of neurotoxic side effects may be caused by high concentrations of a drug, or altered receptor sensitivity to it. To consider these alternatives we established a general relation between cerebrovascular permeability and the octanol/water partition coefficient of a drug. We also formulated a kinetic model that incorporates the observed relation between permeability and partition coefficient, and that can be used to predict brain concentration of a psychoactive drug from the dosage and plasma concentration.

We used our pharmacokinetic model and experimental procedure to examine cerebrovascular permeability in protein-deprived rats and in rats subjected to hyperbaric O_2. Permeability to ^{14}C-sucrose, a poorly permeant water-soluble radiotracer, was unchanged in either case. Thus, neither condition, both of which have been thought to alter cerebral blood vessels, does so.

Erythrocin B, a potentially neurotoxic food additive that has been implicated in hyperactivity and minimal brain dysfunction in children, does not enter the brain of conscious adult rats. Its impermeability, demonstrated exquisitely by our procedure, means that its contribution to hyperactivity in children is doubtful.

In biological systems, it often is critical to distinguish between passive diffusion and energy-dependent, active transport. Active transport helps to regulate the environment of a given organ. At the blood-brain barrier, it may contribute to regulation of brain function. With our new theory, we were able to clearly define criteria for establishing active transport in relation to energy-producing chemical reactions in biological systems.

In 1972, we first demonstrated that the blood-brain barrier could be reversibly opened in animals by infusing a hypertonic solution of a water-soluble nonelectrolyte (e.g. urea, mannitol, arabinose) into the internal carotid artery. The effect later was shown to be caused by osmotic shrinkage of cerebrovascular endothelial cells, with consequent widening of interendothelial tight junctions. In the last years, we experimentally refined the osmotic method, and quantified changes of cerebrovascular permeability in relation to infusate concentration and infusion time, and demonstrated the reversibility of the osmotic effect. We showed that, when the barrier is opened osmotically, brain metabolism is transiently stimulated, brain edema is produced and metabolism is uncoupled transiently from cerebral blood flow. Thus, barrier integrity must be maintained continuously for normal cerebral function. In diseases which affect barrier integrity in man, changes in consciousness may be related to these central effects of barrier disruption.

We also employed the osmotic method as a pharmacological tool. We demonstrated in dogs that the method could be used to increase brain uptake of methotrexate, an anticancer agent, to a sufficient extent so as to treat possibly certain sensitive brain tumors. In collaboration with Dr. E.A. Neuwelt, we successfully applied the osmotic procedure to man, and showed with computerized assisted tomography that the blood-brain barrier can be opened in man without producing apparent neurological changes. A clinical Phase I study was initiated to evaluate the osmotic method for the treatment of unilateral brain tumors with methotrexate and other appropriate anti-tumor agents.

C. Transport Systems at the Blood-Brain Barrier.

A controlled ionic composition of brain interstitial fluid is critical for normal neuronal and glial function, neurotransmission and synthetic processes. It is not known, however, to what extent ionic concentrations are regulated by the blood-brain barrier, although many studies point to active transport. Dr. Smith examined chloride transport at the cerebral capillary endothelium in the unanesthetized rat, and found that there was no active transport but only passive diffusional exchange between blood and brain. When plasma chloride was varied over a 64 nmol/l range under steady-state conditions, brain chloride concentration was proportional to the plasma concentrations, and the unidirectional influx of ^{36}Cl into the brain was linearly related to plasma concentration. Cerebrospinal fluid concentrations were, however, independent of plasma chloride. For chloride, at least, the spinal fluid but not the brain is regulated homeostatically.

D. Function of Peripheral Nerve and Muscle.

The blood-nerve barrier consists of the perineurium, which surrounds the nerve, and the endoneural capillaries which are comparable to continuous capillaries in the central nervous system. Little is known about how these barrier sites affect nerve growth and function.

With electron microscopic tracer techniques, Drs. N. Shinowara and M.E. Michel characterized the perineurium of the frog sciatic nerve as a system of concentric layers of flattened cells interspersed by collagen fibers and connected by tight junctions. They showed that the cells had numerous pits and vesicles, and demonstrate with colloidal and ionic lanthanum that the vesicles were connected to the cell surfaces and never formed transcellular channels. They are examining the role of these vesicles in perineural function.

The perineurium of the frog sciatic nerve was removed and mounted in a bath for continuous infusion, so as to examine transperineural transport and electrical properties. Low permeabilities to ^{14}C-sucrose and to Na$^+$, Cl$^-$ and K$^+$ demonstrated that the perineurium successfully isolates the nerve and endoneurium, but the absence of concentration-dependent transport or sensitivity to specific inhibitors also shows that these ions are not transported actively at the perineurium. Perineural permeability to nonelectrolytes increases in proportion to lipid solubility, indicating that passage for the most part is through and not between the perineural cells. Following nerve transection, perineural permeability changes in relation to the decline and return of nerve function. Thus perineural integrity depends on nerve integrity.

We also examined the electrical properties of the isolated frog perineurium. An AC current was passed across the perineurium and the voltage was measured at frequencies from 1 Hz to MHz. The DC resistance of the perineurium was high, 430 ohm cm^2, further indicating that it is a diffusion barrier to ions. Analysis data suggested that there are two capacitances at the perineurium, a small one whose value equals 0.1 μF/cm^2 and which represents the multiple cell layers of the perineurium, and a large one with a value of 20 μF/cm^2 and which probably is due to polarization of intercellular tight junctions that connect some perineural cells. Experimental manipulation of this large capacitance may help us to understand the chemical nature of the junctions.

Dr. Ask examined the compliance of the nerve and the role of the perineurium in responding to edema. Edema is a common consequence of toxic and metabolic insults to nerves. He demonstrated that the hydrostatic pressure in the endoneurium is positive, and that the low permeability of the perineurium makes it act as an osmotically responsive, semi-permeable membrane.

We prepared data for publication on muscle metabolism in relation to muscle fatigue. Fatigue of single fibers from the frog semitendinosus muscle appeared as a decline in tension output during prolonged tetanic stimulation, and occurred even though significant quantities of ATP, the energy source for muscle contraction, remained. Caffeine, when applied to fatigued fibers, produced contractures and at the same time depleted residual ATP concentrations. Thus, the ATP was not sequestered and was available for contraction. From our measurements of fiber lactate, we suggested that H$^+$ accumulated in the fatigued fibers so as to uncouple excitation from contraction. Uncoupling may be a protective mechanism to prevent total depletion of energy stores, which would result in irreversible muscle contracture and cell death.

E. Drug Pharmacokinetics, Relation to Pharmacodynamics and Senescence.

The elderly respond differently to centrally-acting drugs than do mature adults. They are more susceptible to untoward sedation with benzodiazepines and to extrapyramidal symptoms with neuroleptics, drugs which are widely prescribed for them. Only limited information is available on the causes for the altered responses.

Dr. I. Kapetanovic examined age-dependent responsivity to haloperidol in the Fischer-344 rat, in terms of pharmacokinetics (factors which affect drug metabolism and organ distribution) and pharmacodynamics (factors which relate to brain responsivity to a drug or its metabolites). He developed analytical methodology to measure brain and plasma haloperidol concentrations, when using tissue extraction techniques and gas chromatography with a nitrogen selective detector. His data were analyzed in terms of mathematical models for biodisposition. Following an intraperitoneal bolus injection of 0.5 mg/kg haloperidol, plasma elimination half-life was 3.6 hr in 3-month old male Fischer-344 rats, and 2.6 hr in 33-month-old, senescent animals. After 6 and 9 hrs, plasma concentrations were 2-fold higher in senescent animals, as were brain concentrations. However, steady-state studies with continuous i.p. infusion showed no age-related differences. The cause for an age-related difference in the acute pharmacokinetic response but not in the steady state response to haloperidol remains to be examined.

F. Central Nervous System Binding Sites for the Neurotoxin Kainic Acid.

Dr. E. London examined central nervous system binding sites for the neurotoxin, kainic acid, which appears to be specific for neurons that contain glutamic acid receptors. She demonstrated binding sites in human brain autopsy material, with kinetic characteristics similar to binding sites in the rat, and thus demonstrated that kainate's neurotoxicity was relevant to human disease. She showed that high-affinity binding sites were reduced in the caudate nucleus and putamen of the human brain in Huntington's disease. Their reduction supports the concept that the pathogenesis of Huntington's disease involves an imbalance in neurotransmitter systems which interact with afferent nerves that release glutamate, a central neurotransmitter, within the basal ganglia.

G. Pharmacology of Central and Peripheral Catecholaminergic Systems.

Dr. C.C. Chiueh examined the cardiovascular status and plasma catecholamine and cortisol concentrations of Fischer-344 rats in relation to age, under resting and immobilization conditions. Resting Fischer-344 rats underwent a decrease in heart rate with age, although plasma norepinephrine increased. Furthermore, older rats did not respond with the blood pressure changes and rapid catecholamine release of younger animals. They had a reduced responsivity to adrenergic stimulation in both the resting and stressed states.

Corresponding to the age-related increase in circulating plasma catecholamines, there is growth of extra-adrenal chromaffin tissue in paraganglia of the Fischer-344 rat. Dr. M. Partanen showed that paraganglia are located close to sympathetic ganglia and the aorta, degenerate postnatally, but appear again with senescence, and contain large quantities of catecholamine-containing cells in 33-male old Fischer rats.

Local glucose utilization was measured in several sympathetic ganglia of the Fischer-344 rat in relation to age, to estimate functional activity. The 2-deoxy-D-glucose technique of Sokoloff (see below) was employed. Between 12 and 24 months of age, a statistically significant increase in glucose utilization occurred in the superior cervical ganglion. The results suggest that functional activity of the sympathetic nervous system increases with age, possibly in partial compensation for decreased responsivity of peripheral receptors to circulating catecholamines.

H. Assessment of Neurochemical Markers in Relation to Age and Behavior.

In related mouse strains, age-associated changes in several neurochemical markers such as neurotransmitter synthetic enzymes appear to be influenced by genetic variability. Dr. S. Waller and E. London examined three enzymes that are responsible for the synthesis of critical neurotransmitters, of which the function and concentration might be related to animal behavior. The enzymes were choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TH), responsible for the synthesis, respectively, of gamma-amino butyric acid, catecholamines and acetylcholine. The enzymes were measured in 4 brain regions of C57BL/6J and A/J mice strains, characterized by differences in activity and learning ability.

There was an approximately 30% increase in cerebellar TH activity in both strains between 4 and 24 months of age. There also were several age-associated alterations in enzyme activity that were strain-related. An example is GAD activity in the striatum between 4 and 24 months. It increased in C57BL/6J mice and decreased in A/J mice. In addition, normal mature C57BL/6J mice when compared to A/J mice, had higher TH and CAT activities in all four brain regions, higher cortical and cerebellar GAD activity, and lower hippocampal and striatal activity. The studies demonstrated that brain synthetic neurotransmitter enzymes are strain-related in mature animals, and that their pattern of change with aging depends on genotype. As these enzymes have also been measured in man, and shown to be affected in certain diseases (e.g. a reduced CAT activity in Alzheimer's disease), their characterization in relation to behavior and genotype should help to elucidate their role in cognition and behavior in man.

I. Cerebral Metabolism, Relation to Brain Function and Aging.

Regional cerebral glucose utilization and regional cerebral blood flow reflect the functional state of the brain. Dr. E. London employed the ^{14}C-2-deoxy-D-glucose method of Sokoloff et al. (1977) to examine regional cerebral glucose utilization in Fischer-344 rats at 1,3,12,24 and 34 months of age. She wanted to evaluate age-related alterations in functional states in specific brain regions, because of published studies on age-related changes in neurotransmitter systems. Regional glucose utilization increased between 1 and 3 months of age, when the rat brain continues to grow, but then, remarkably, fell between 3 and 12 months. The cause of this fall is not known, but may be related to neuroendocrine changes. Glucose utilization did not change further after 12 months of age in the resting rat. This indicates that resting glucose utilization is not a measure of the many functional changes which occur during senescence. Pharmacological or physiological stress may be necessary to demonstrate defective cerebral function with the 2-deoxy-D-glucose technique.

Dr. M. Ohata reported changes in local cerebral blood flow in the brain of the unanesthetized Fischer-344 rat at ages of 1,3,12,24 and 34 months. Blood flow, as proposed by Roy and Sherrington (1890), responds to functional demands and under normal conditions is a dynamic measure of cerebral activity. ^{14}C-iodoantipyrine was used to measure flow. Blood flow, like glucose utilization (see above), increased in most brain regions between 1 and 3 months of age, but continued to increase from 3 to 12 months when glucose utilization simultaneously fell. Thus, there was a dissociation in the time courses of cerebral flow and metabolism in relation to aging. Changes in blood flow after 12 months of age probably reflect function deafferentation of specific brain regions.

Dr. H. Takei measured, for the first time in conscious rats, the cerebral metabolic rates for O_2 ($CMRO_2$), glucose (CMR_{glc}) and CO_2 ($CMRCO_2$), as a function of age. This procedure involved a two-step operation that required catheterizing the superior sagittal sinus. The parameters reflect mainly oxidative metabolism of the cerebral cortex. He demonstrated that, between 3 and 24 months of age, there were no statistically significant declines in any of the parameters, and thus, despite known neurochemical and morphological changes that occur in the aging brain, cerebral cortical oxidative metabolism does not decline significantly. The combined studies on local and global measures of cerebral cortical oxidative metabolism suggest furthermore that plasticity responses are sufficient in the aging brain to maintain normal functional activity under resting conscious conditions.

Immobilization of rats is reported to increase cerebral metabolism and blood flow by more than 100%. As many of our studies are performed on partially immobilized conscious animals, we wanted to examine the effect of immobilization in this preparation. Dr. M. Ohata found that immobilization did not increase blood flow, contrary to prior studies, and that slight decreases that occurred were due to hyperventilation and reduced arterial CO_2.

Dr. N. Shinowara showed that glucose utilization increases between 1 and 3 months in the retina of the Fischer-344 rat, but then declines in relation to the gradual loss of photoreceptors due to both aging and light damage. Glucose metabolism decreases only slightly, however, at the superior colliculus (part of the central visual pathway), despite retinal degeneration with age.

Dr. E. London initiated two projects, one with a muscarinic cholinergic agonist (oxotremorine), the other with a series of GABAergic drugs, to examine their specific dose-related effects on the central nervous system of Fischer-344 rats. In the conscious rat, oxotremorine stimulated glucose utilization in many brain regions, but not all of them (e.g., red nucleus) have high levels of receptors for acetylcholine. The effect of the drug was specific and could be blocked by prior administration of scopolamine, which crosses the blood-brain barrier, but not of atropine methyl bromide, which does not. Dr. London and colleagues also mapped out specific brain regions that have a markedly increased metabolic rate following administration of the GABAergic drugs, muscimol and bicuculline. Because the increase was not clearly related to receptor density, pharmacological activation of the central nervous system probably depends on neuronal input as well as receptor density.

J. Brain Function in Aging and Disease in Man.

Behavioral and cognitive performance in normal man at different ages was examined in relation to local cerebral glucose utilization by Drs. R. Margolin, R. Duara and E. Robertson-Tchabo. Male subjects in 3 age categories, 20-30 years, 40-50 years and 65+ years, were screened by medical diagnostic procedures and by personality and psychometric tests to exclude secondary or primary brain disease. These normal volunteers then were submitted to positron emission tomography (PET scanning), following the intravenous injection of ^{18}F-2-deoxy-D-glucose, for the measurement of regional rates of cerebral glucose utilization. Brain radioactivity was determined at different times after injection. Ten successful scans were completed. The data are being analyzed and related to parameters of brain function.

One normal man, age 59, was found with a 20% reduction in glucose utilization at the right parietal lobe. Regional cerebral blood flow as measured by inhalation of ^{133}Xe, also was reduced at the right parietal lobe. As cognitive or neurological defects were absent, as far as we could measure, the results serve as a cautionary note to PET scan users who, for example, in examining cognitive or psychiatric defects in disease states such as schizophrenia, might interpret an isolated reduction in glucose utilization as demonstrating a brain region which is related to the disease process.

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NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00120-04 LNS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Blood-Brain Barrier and Central Nervous System Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new method was developed to measure cerebrovascular permeability to poorly permeable as well as rapidly permeable drugs. Pharmacokinetic principles for the central nervous system were established that should make it possible to calculate brain concentrations of drugs from measured plasma concentration curves. Furthermore, new criteria were established to evaluate the presence or absence of active transport at biological membranes. It was shown that cerebrovascular integrity was not changed in protein-deprived rats, that hyperbaric O_2 treatment does not alter the blood-brain barrier, and that Erythrocin B, a potentially neurotoxic food additive, does not enter the brain of conscious rats. Osmotic opening of the blood-brain barrier was characterized in rats by autoradiographic procedures, and was successfully employed in humans with metastatic brain tumors to allow methotrexate into the brain. Neurotoxicity did not appear clinically, when cerebrovascular integrity was monitored with computer tomography and radionuclide brain imaging. In conscious animals, blood brain barrier opening by osmotic or hypertensive means increased local cerebral glucose utilization.

PHS-6040
(Rev. 2-81)

Objectives: The blood-brain barrier at the cerebral vasculature regulates the ionic environment of the nervous system, has specific mechanisms for transport of amino acids and glucose that support cerebral metabolism and neurotransmitter and protein synthesis, and prevents access to the brain of water-soluble drugs and other agents. The aims of this project are as follows: (1) to describe the ultrastructure of the blood-brain barrier in relation to its permeability and physiologic functions in the normal and aging brain, (2) to determine the quantitative rules that govern drug entry into the brain, (3) to develop a method to reversibly modify barrier permeability, and to apply it to understanding the central actions of systemically-administered drugs and other agents, and (4) to determine how the barrier regulates cerebral metabolism and behavior.

Methods Employed: A number of different methods were employed in these studies. Surgical procedures were used to open the skull and dura of anesthetized animals, and to catheterize blood vessels for infusion and plasma sampling. Neurological examinations and behavioral tests evaluated overall brain function and localized deficits. Histological methods were used to examine brain pathology, and electronmicroscopy was employed to evaluate transfer mechanisms at the barrier and barrier ultrastructure. Radioisotope techniques including autoradiography were used to quantitate drug and metabolite transfer from blood to brain. Computers were used to analyze data and to generate relevant models.

Major Findings: 1. Blood-Brain Barrier.

a. Quantitative Aspects of Drug Entry into the Central Nervous System.

(1) Central nervous system pharmacokinetics and cerebrovascular permeability. The blood-brain barrier limits exchange of drugs between plasma and brain, and makes it difficult to predict dose-response relations for centrally-acting drugs. We therefore elaborated a pharmacokinetic model to interpret brain-blood-cerebrospinal fluid exchange, and developed quantitative experimental methodology to determine exchange in the unanesthetized rat. A radiotracer drug is injected intravenously, arterial plasma radioactivity is determined periodically thereafter, until the animal is killed and brain radioactivity is measured. The cerebrovascular permeability-area product, PA sec^{-1}, is calculated as the ratio of brain concentration (after correction for intravascular radioactivity) to the plasma concentration integral. Experiments are limited to times in which back diffusion from brain to plasma is insignificant (Ohno, K., Pettigrew, K.D., and Rapoport, S.I.: Amer. J. Physiol. 253: H299-H307, 1978). The method is 100 times more sensitive than other available methods and was used to evaluate cerebrovascular integrity in protein-deprived rats, and in rats exposed to hyperbaric O_2, and to measure PA of Erythrocin B in conscious rats (see below).

The model was extended to take into account exchange among plasma, cerebrospinal fluid and the brain intracellular and extracellular compartments. The equations derived with the extended model were used to analyze ^{14}C-urea pharmacokinetics, to obtain reliable transfer constants among the different cranial compartments. Once transfer constants of a particular drug are known, the model can be used to predict brain concentrations from any measured plasma and cerebrospinal fluid concentration curves.

(2) Cerebrovascular integrity in protein-deprived rats. Protein deprivation causes a large number of alterations in the central nervous sytem of maturing experimental animals. It appeared possible that some of these changes are related to defective functioning of the blood-brain barrier. An alteration of barrier permeability has been suggested to contribute to increased susceptibility of fat-deprived rats to experimental allergic encephalitis (Selivonchick *et al*., J. Nutrition 105: 288-300, 1975). We therefore measured cerebrovascular permeability to ^{14}C-sucrose, a non-metabolizable, poorly penetrating water-soluble tracer, in protein deprived rats. This tracer crosses the blood-brain barrier slowly, and even small alterations in capillary integrity would be evidenced by increased permeability. We found, however, that protein deprivation did not increase ^{14}C-sucrose permeability at the blood-brain barrier in rats aged 3.5 to 12 months. PA, the product of cerebrovascular permeability (P) to ^{14}C-sucrose and capillary surface area (A), was very low in mature rats that had been maintained either on an 8% or 25% casein diet, and equaled 8×10^{-6} sec^{-1} in both groups (Ref. 1).

(3) Lack of hyperbaric 0_2 effect on blood-brain barrier permeability in conscious rats. Periodic exposure of rats or cats to hyperbaric 0_2 has been reported to increase blood-brain barrier permeability to intravenously injected cadmium-free ferritin and human placental β-hexosaminidase (Lanse *et al*., Aviat. Space Environ. Med. 49: 890-894, 1978; Rattazzi *et al*., Second. Intl. Symp. Enzyme Therapy and Genetic Disease. R.J. Desnick, Ed. Birth Defects: Original Article Series 16, 179-193, 1980. Alan Liss, N.Y.). Animals treated for 90 min each day with 100% 0_2 at 2.5 ATA, over 5 consecutive days, are reported to show cerebral extravasation of blood-borne proteins. As the treatment has been suggested as a potential clinical tool in enzyme replacement therapy in man, we thought it important to confirm the findings with a quantitative radiotracer technique, as developed by Rapoport *et al*. (Brain Res. 150: 653-657, 1978). Conscious rats were exposed to 100% 0_2 at 2.5 ATA for 90 min for up to 10 consecutive days, or to a N_2-0_2 mixture (PO_2 = 0.3 ATA) under the same conditions (control animals). Cerebrovascular permeability to ^{14}C-sucrose, was not altered by hyperbaric 0_2 exposure in the experimental animals as compared to controls. The results differ with reports of increased permeability in response to hyperbaric 0_2 (see above), and show that hyperbaric 0_2 is not of potential use for clinical modification of blood-brain barrier integrity (Ref. 2).

(4) Lack of entry of Erythrocin B into central nervous system. Food color additives have been held partly responsible for behavioral disorders such as hyperactivity and minimal brain dysfunction in children. At least one widely used food color, Erythrocin B (FD & C Red No. 3), can change neuronal membrane physiology, neurotransmitter release and uptake, and the activity of brain Na-K-ATPase when applied directly to cells or subcellular organelles. There is no information, however, on whether significant levels of this dye appear in the brain after consumption or administration. In order to determine whether Erythrocin B can cross the blood-brain barrier and enters the brain in detectable amounts, we injected ^{14}C-Erythrosin B intravenously into unanesthetized male mature (3 month-old) Osborne-Mendel rats. The tracer was injected either alone or 10 minutes following intravenous injection of a saturating concentration of

nonradioactive dye. Blood radioactivity was followed, and the brain was removed 10 min, 30 min or 2 hr after injection. Brain radioactivity was measured, after correction for intravascular tracer. In other experiments, ^{14}C-Erythrocin B and ^{3}H-sucrose were infused directly into the carotid artery of rats about 1 min after the ipsilateral cerebral hemisphere had been perfused with a saline solution to remove blood. These animals were decapitated 1 minute later and their brains were removed. In none of the 14 brain regions examined did the concentration of Erythrosin B significantly exceed that of sucrose, a substance used as an indicator of brain blood volume because it penetrates the blood-brain barrier poorly. We conclude that intravascular Erythrocin B does not enter the brain of mature rats in measurable amounts. An abstract of this work has been published (Levitan, H., Ziylan, Z., Smith, Q.R., and Rapoport, S.I. Abstr. Eleventh Annu. Meeting Soc. Neurosci. 1981).

(5) Characteristics of active transport and passive diffusion across biological membranes. Radiotracers have been employed to examine permeability and transport of material across natural membranes. Although passive diffusional movement is determined by the electrical potential (for ions) and concentration gradient, active transport, which characterizes biological systems, is determined in part by the free energy of the chemical reaction to which the transport is coupled. H. Ussing (Acta Physiol. Scand. 17: 1-37, 1949) introduced criteria by which the ratio of unidirectional fluxes across a membrane can be used to demonstrate active transport. We extended his theoretical analysis.

For chemical reactions not at equilibrium, but proceeding in the forward direction at the steady state, the unidirectional flux ratio $\vec{j}/\overleftarrow{j} \leq \exp(-\Delta G/RT)$ where R ΔG is the free energy of the reaction, the gas constant and T is absolute temperature. The conditions under which the equality applies were derived. If unidirectional fluxes are not in the steady state, the flux ratio is time invariant under certain situations. One important case is for systems with an ordered sequence of reactions. For systems with more than one pathway, $\vec{j}/\overleftarrow{j}$ is not constant except for special cases. The analysis helps to clarify the conditions necessary for distinguishing active transport from passive diffusion in biological membranes (Ref. 3).

b. Reversible Modification of Blood-Brain Barrier Permeability.

(1) Methods and mechanism. The blood-brain barrier at cerebral capillaries is due to a continuous layer of endothelial cells that are connected by tight junctions. We demonstrated that the barrier can be opened reversibly by infusing a hypertonic solution of a water-soluble solute (e.g. arabinose or mannitol) into the carotid artery of animals or man. We also showed, with electronmicroscopy and by theoretical models, that barrier opening probably is caused by shrinkage of cerebrovascular endothelial cells and widening of interendothelial tight junctions. The method should prove useful as a tool for studying central actions of drugs that normally do not penetrate the blood-brain barrier.

(2) Transient functional and metabolic consequences.

Osmotic barrier opening can be accomplished in animals without producing long-term changes in behavior, brain water or electrolyte content or brain histology. However, if the procedure is employed in conscious rats, then transient changes occur that are reversed within 2 to 24 hr. Brain water is elevated by about 1%, due to barrier opening to salts and loss of the protective barrier action against brain edema, and cerebral blood flow no longer is tightly coupled to cerebral metabolism. Finally, behavioral and cerebral metabolic changes reminiscent of convulsions (some changes are prevented by Valium) are produced by barrier opening. Regional uptake of ^{14}C-2-deoxy-D-glucose is increased. This tracer is a glucose analogue that is transported and phosphorylated like glucose within the brain but not metabolized further.

The metabolic stimulation that is evidenced by increased brain uptake of ^{14}C-2-deoxy-D-glucose, following osmotic barrier opening in the rat, is not caused by stimulation of central α- or β-adrenergic receptors, because it is not prevented by prior administration of either phenoxybenzamine (an α-adrenergic blocker) or propranolol (a β-adrenergic blocker). Furthermore, it is not due to entry of potassium from plasma to brain, with consequent neuronal activation, because it occurs in potassium-deprived animals in which plasma potassium is reduced below brain extracellular potassium. Metabolic stimulation does occur after hypertensive as well as osmotic barrier opening, however, and therefore is not specific to the barrier insult. The cause at present is not known. Such stimulation, which probably represents local convulsive activity and is accompanied by a reduced cerebral blood flow, may contribute to changes consciousness in maladies of the central nervous system in which the blood-brain barrier is disrupted (e.g. Alzheimer's disease, multiple sclerosis, hypertensive encephalopathy). An abstract of this work has been published (Rapoport, S.I. Special Lecture. Pathological alterations of the blood-brain barrier. Abstracts 8th. Annual Meeting, Soc. Neurosurgical Anesthesia and Neurological Supportive Care. St. Louis, MO, 1980, pp. 1-9).

(3) Quantification and time course. In order to apply the osmotic method as a pharmacological tool, we determined thresholds for infusate concentration and infusion time that produce maximum opening, as well as the time course of such opening. To do this, we used ^{14}C-sucrose as an intravascular tracer to measure and quantify osmotically-induced cerebrovascular permeability changes in the rat (Rapoport et al., Amer. J. Physiol. 238: R421-R431, 1980).

This methodolgy was extended to determine regional cerebrovascular permeability in animals by means of autoradiography. In this case, ^{14}C-aminoisobutyric acid, a poorly permeant tracer that is taken up by brain cells if allowed to enter the brain, was injected intravenously in rats following carotid infusion of 1.6 molal arabinose solution. Plasma concentrations were followed until decapitation, and brain distribution of tracer was determined by autoradiography of frozen slices. The results confirmed the findings that the blood-brain barrier can be opened by osmotic treatment in rats, and established the cerebral distribution of the osmotic barrier effect (Ref. 4).

In order to eventually apply the osmotic method to facilitate drug entry into the brain of man, we developed an in vivo method to monitor blood-brain barrier disruption, using computerized axial tomography. In the dog, a hypertonic mannitol solution was infused into the internal carotid artery, following the intravenous injection of Conray 60 as a radiocontrast agent. Immediately after osmotic treatment, methotrexate was infused through the carotid circulation. Computerized tomograms of the brain demonstrated reversible osmotic blood-brain barrier disruption. These *in vivo* measures of disruption correlated with brain uptake of methotrexate, an anti-tumor drug that normally enters the central nervous system very slowly (see below) (Ref. 5,6).

(4) *Pharmacological uses of reversible osmotic barrier opening*. Osmotic barrier opening was employed to allow the following normally-excluded agents into the brain:

(a) *Methotrexate, for therapy of brain tumors*. One of the major problems in cancer chemotherapy is the inability of systemically effective drugs to act on metastatic brain tumors, because of the blood-brain barrier. It would be extremely important, especially in cases of single brain metastases in association with pulmonary or breast carcinoma, to be able to open the blood-brain barrier so as to allow these agents into the brain.

Carotid infusion of hypertonic mannitol solution first was used to open the blood-brain barrier to methotrexate in dogs. Ipsilateral methotrexate brain levels ranged from 90-216 ng/g tissue following intraarterial injection of drug in control animals, whereas a mean value of 1420 ng/g was measured after mannitol treatment. Brain levels remained high for 6 hrs. There were no neuropathological changes 24 or 72 hrs after infusion, or up to 2 weeks later. The methotrexate levels reached under these conditions would be sufficient to treat certain cerebral tumors (Ref. 5,6).

(b) *Reversible blood-brain barrier disruption in man in chemotherapy of malignant brain tumors*. Successful experimental reversible disruption of the blood-brain barrier in animals provided the basis for a clinical evaluation of osmotic disruption in five patients with primary and metastatic malignant brain tumors. Reversible, transient osmotic barrier disruption was achieved 15 times in the 5 patients without additional toxicity, using 25% mannitol which was infused into the internal carotid artery *via* a catheter placed in the femoral artery. Computed tomography and radionuclide brain imaging were shown to be useful noninvasive monitors of the adequacy and extent of barrier disruption. These studies provided further evidence that the barrier is at least partially intact in human tumors, because one patient demonstrated a cerebral metastasis only after barrier disruption. These studies were performed in collaboration with Dr. E. Neuwelt (Ref. 7).

Significance to Biomedical Research and to the Program of the Institute :

1. An understanding of how the blood-brain barrier regulates the brain environment and controls brain metabolism is critical for interpreting changes of brain function in health, aging and disease. Our findings establish the ultrastructural and transport properties of the blood-brain barrier.

2. Our methods in conscious rats, together with pharmacokinetic theories that we are developing, make it possible to measure cerebrovascular permeability of drugs

as a function of their physical properties, and to measure transfer constants between blood and brain compartments. It should be possible, on the basis of the findings, to predict and interpret dose-response relations of centrally-acting drugs in man and animals. An altered response in relation to age could be due to an altered rate of drug entry into the brain or to changes in cerebral receptor sensitivity.

3. Our findings of normal cerebrovascular integrity in protein-deprived rats indicate that brain changes caused by protein deprivation are not due to altered blood-brain barrier function. The lack of a hyperbaric O_2 effect on cerebrovascular integrity demonstrates that a prior reported positive effect probably is erroneous. It suggests that hyperbaric O_2 treatment would not be useful in man for increasing uptake of drugs or enzymes by brain.

4. Erythrocin B has been implicated in hyperactivity and minimal brain dysfunction in children, and can modify neuronal function. It is included as a dye in many food·stuffs. However, our finding that it does not enter the brain of the mature rat to a significant extent suggests that, when the blood-brain barrier is intact, it should have no influence on brain function.

5. Reversible osmotic opening of the blood-brain barrier is the first and only method that allows normally excluded agents into the brain without producing long-term brain damage. Now that the method has been shown to be feasible in man, it probably will be employed more generally for treatment of brain tumors, for enzyme replacement therapy and for augmenting drug entry into the brain in cerebral infections. The human findings set the stage for serious clinical trials with given end points for effective therapy.

6. Our finding that osmotic and hypertensive barrier opening increase local cerebral glucose utilization by the brain, reduce cerebral blood flow and produce brain edema demonstrate that an intact blood-brain barrier is necessary for the maintenance of normal cerebral metabolism and function, coupling between cerebral blood flow and metabolism, and prevention of brain edema. Changes in consciousness in central nervous system diseases that may involve blood-brain barrier damage (e.g. multiple sclerosis, hypertensive encephalopathy) may be related directly to altered cerebrovascular permeability.

Proposed Course: (1) The pharmacokinetic rules that govern drug transfer from plasma to brain compartments will be elaborated, and models will be developed and tested to interpret dose-response relations of centrally-acting drugs in animals and man, (2) further application of the osmotic method will be made in human and animal studies, for its eventual use as a pharmacological tool, (3) we will try to determine why changes in blood flow and metabolism accompany opening of the blood-brain barrier, and (4) further ultrastructural studies will be performed to define the basis of increased cerebrovascular permeability following osmotic treatment.

Publications:

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5. Neuwelt, E.A., Frenkel, E.P., Rapoport, S.I. and Barnett, P.: Effect of osmotic blood-brain barrier disruption on methotrexate pharmacokinetics in the dog. Neurosurgery 7: 36-43, 1980.

6. Neuwelt, E.A., Frenkel, E.P., Diehl, J.T., Maravilla, K.R., Vu, L.H., Clark, W.K., Rapoport, S.I., Barnett, P.A., Hill, S.A., Lewis, S.E., Ehle, A.L., Beyer, C.W., Jr. and Moore, R.J.: Osmotic blood-brain barrier disruption: a new means of increasing chemotherapeutic agent delivery. Trans. Amer. Neurol. Assoc. 104: 1-5, 1980.

7. Neuwelt, E.A., Frenkel, E.P. Diehl, J., Vu, L.H., Rapoport, S.I. and Hill, S.: Reversible osmotic blood-brain barrier disruption in humans: implications for the chemotherapy of malignant brain tumors. Neurosurgery 7: 44-52, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00121-04 LNS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Function of Peripheral Nerve and Muscle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A. The perineurium of frog sciatic nerve, which regulates nerve function, contains concentric cell layers that act as a diffusion barrier because of the presence of tight junctions. The cells have vesicles which take up extracellular lanthanum but do not form complete transcellular channels. Perineurial permeability is low to ions and water-soluble nonelectrolytes, but high to lipid soluble nonelectrolytes. There is no evidence of active ion transport. During Wallerian degeneration, perineurial permeability changes in relation to nerve function. The hydrostatic pressure in the endoneurium is positive and reflects the role of the perineurium as an osmotically active membrane.

B.. Fatigue of single frog muscle fibers appears as a decline in tension output during prolonged tetanic stimulation. The concentration of ATP (energy source for contraction) remained normal in fatigued fibers. Furthermore, ATP is not unavailable for contraction, because it can be consumed in response to caffeine. We suggest that H^+, as lactic acid, accumulates in fatigued fibers so as to interfere with excitation-contraction coupling.

The object of this project is to relate metabolism and morphology of peripheral nerve and muscle to the functioning of these tissues in health, disease and aging.

1. Peripheral nerve and perineurium. Peripheral nerve function is governed in part by the local axonal milieu, which is separated from body fluids by a perineurial sheath and endoneural capillaries. We studied the function and morphology of the perineurium, in order to understand the role of the perineurium in regulating the endoneural environment of axons. The ability of the perineurium to act as a permeability barrier to radiotracer nonelectrolytes and to ions was examined in normal, degenerating and regenerating nerves. The hydrostatic pressure, and volume of the endoneural space, were measured in relation to fluid flow through the perineurium.

2. Muscle contraction and muscle fatigue. When a striated muscle is stimulated repetitively, its contractile force decreases and it becomes fatigued. Fatigue characterizes fast twitch muscles with glycolytic metabolism, rather than slow twitch muscles with oxidative metabolism. Muscle fatigue generally is thought to be caused by depletion of available energy reserves. However, studies in single fibers suggest that fatigue can be due to uncoupling of steps that connect excitation with contraction. We explored the relation between fatigue and metabolism in single muscle fibers rather than in whole muscles, because, in whole muscles, some fibers can fatigue faster than others and measured metabolite concentrations are averaged over a heterogeneous, often disparate fiber population.

Methods Employed:

1. Perineurium. For electrical and radioisotope flux studies, the perineurium of the frog sciatic nerve was isolated and mounted on cannulae within a bath of stirred Ringers solution, and perfused by Ringers solution. Radioisotopes were placed in the bath. Transport through the sheath was quantified by measuring isotope entry into the perfusate.

The AC impedance of the perineurium of the frog sciatic nerve was measured *in vitro* using a four electrode arrangement, where a sinusoidal current of varying frequency (1 Hz to 0.1 MHz) was imposed across the perineurium. This current was compared with the resultant transperineurial potential difference.

To cause Wallerian degeneration, the right sciatic nerve was transected in ether-anesthetized frogs, with the left serving as control. The compound action potential and the sodium (22Na) permeability of the perineurium of the distal segment of the nerve were examined 3 to 42 days after transection, and compared to observations in the control nerve.

For morphological studies, the frog sciatic nerve was fixed with glutaraldehyde *in situ* or *in vitro* by immersion. Rapid preservation was achieved by using an osmium tetroxide-glutaraldehyde fixative. Colloidal lanthanum or lanthanum chloride was presented for 3 to 4 hours externally to small segments of nerve, or internally by injection. Horseradish peroxidase was used as an in vitro tracer in the bath for one hour at 5° or 25°C. Tissues were prepared for electron microscopic examination.

Endoneural hydrostatic pressure was modified in isolated frog sciatic nerves by immersing them in solutions with different osmolarities. Endoneural pressure measurements were made with microelectrodes, connected to a null-balance feedback system. Endoneural volume was calculated from nerve diameter.

2. Muscle. A single fiber was dissected from the frog semitendinosus muscle and mounted in a bath of flowing Ringers solution at 15°C. Tension was recorded by a RCA 5734 transducer tied to one end of the fiber; the output was displayed on an oscilloscope face. Sarcomere length was measured by a laser diffraction technique. The fiber was stimulated at different frequencies via external platinum electrodes, so as to produce twitches or tetanic contractions.

Major Findings:

1. Perineurium and Peripheral Nerve

a. Morphology of perineurium. The perineurium of the frog sciatic nerve consists of 7 to 19 concentric layers of flattened cells, interspersed by collagen fibers. Close membrane appositions of tight junctions were observed between cells throughout the layers. As shown with electronmicroscopy, the flat cells had numerous pits and vesicles at or near their surface membranes. Lanthanum (coloidal or ionic) when applied to the nerve in vitro, was seen in all layers of the perineurium, down to the inner "barrier" layers, but never within the endoneurium. In areas of lanthanum penetration, tracer was observed in the extracellular space laterally for long distances, between unconnected ends of cellular processes, and within vesicles attached to surface membranes. Vesicles appeared as small round-bottomed or oval flasks extending from both cell surfaces. Occasionally a filled vesicle appeared free within the cytoplasm. When specimens were tilted, however, several variations in vesicle shape were noted. Flasks which had seemed to fuse with the opposite membrane to form a channel were actually unattached. "Free" vesicles were either attached by necks to the cell surface or formed clusters with other flasks. Although some chains or clusters of vesicles were observed, they were attached to only one surface membrane. Under these experimental conditions, therefore, a large number of vesicles, connected to the surface membranes, appear at the inner cellular layer of the perineurium. However, vesicle fusion and transcellular channels do not significantly contribute to the passage of material between perineurial layers. An abstract of this work has been published (Shinowara, N.L., Michel, M.E. and Rapoport, S.I. Abstr. Eleventh Annu. Meeting Soc. Neurosciences, 1981).

b. Permeability and transport of perineurium. The permeability of the perineurium of the frog sciatic nerve to ^{22}Na, ^{42}K and ^{36}Cl equaled 1.7, 2.9 and 2.9 x 10^{-6} cm/sec, respectively. Oxygenation, addition of glucose to the bath, or applications of inhibitors of active ion transport, such as ouabain and amilorid, did not significantly alter ion fluxes across the perineurium in either the inward or outward direction. This implied that these ions are not actively transported and that the perineurium does not actively regulate the nerve ionic environment. The low ionic permeabilities make the perineurium a substantial barrier to ion exchange between nerve and tissue extracellular space.

The nonelectrolyte permeability pattern of the isolated frog perineurium further showed that the perineurium is a significant diffusion barrier to water-soluble substances. Nonelectrolyte permeability ranged from a low value of 5.6×10^{-7} cm/sec for ^{14}C-sucrose to 2.7×10^{-4} cm/sec for ^{14}C-n-butanol. Nonelectrolyte permeability increased in proportion to lipid solubility, as measured by the octanol/water partition coefficient. The order of permeabilities is as follows: sucrose < mannitol < glycerol < thiourea < ethylene glycol < urea < DMSO < caffeine < water < n-butanol.

c. Endoneural hydrostatic pressure and perineurial hydraulic conductivity. When segments of the sciatic nerve of the frog were immersed in hypertonic or hypotonic Ringer solutions, nerve volume and endoneural hydrostatic pressure decreased linearly with the reciprocal of bathing solution osmolarity. The linear relation provided a value for nerve compliance. A mathematic model was developed to analyze the pressure-volume relation in terms of internal osmolality, perineurial hydraulic conductivity, and tissue compliance. Hydraulic conductivity was calculated to be low and characteristic of tight epithelial tissues that act as significant barriers to water and ion flow.

d. Electrical properties of perineurium. The DC resistance of the perineurium was 430 ohm cm^2. Impedence measurements demonstrated two dispersions with center frequencies of 5 KHz and 20 Hz. Exposure to high conductance Ringer solution decreased the DC resistance, whereas a low conductance Ringer increased the resistance. Analysis of the data in terms of a four-element equivalent circuit (two resistors and two capacitors) suggested that small (0.1 μF/cm^2) and large (20μF/CM^2) capacitances exist at the perineurium. The small capacitance was ascribed to six or more layers of perineural cells. The large capacitance was ascribed to polarization of charge, perhaps at intercellular tight junctions, and was affected by experimental manipulation. An abstract of this work has been published (Weerasuriya, A., Spangler, R.A., Rapoport, S.I., and Taylor, R.E., Electrical impedance of the perineurium of the frog sciatic nerve. Biophys. J. 25: 300a (1979)).

e. Wallerian degeneration. Following proximal transection of the frog sciatic nerve, perineural permeability increased when the amplitude of the compound action potential decreased during Wallerian degeneration of the nerve. Three days after transection there was no significant change in either perineural permeability or the compound action potential, but by 6 weeks the compound action potential was absent, and perineurial ^{22}Na permeability had increased more than eight-fold. From the third to the sixth day after nerve transection, the permeability increased gradually, with the greatest changes occurring between the 14th and 21st day. On the other hand, the sharpest decrement in compound action potential was between the 10th and 14th day. The results show that perineurial integrity depends on the integrity of the nerve itself (Ref. 1).

2. Muscle. We first distinguished the time course of the fatigue process in single fibers from post-tetanic potentiation of the twitch, which is characterized by elevation and prolongation of the twitch and is due to intracellular calcium accumulation. Fatigue appeared after 20 sec of tetanic stimulation (at 20 Hz) as a decline in tetanic tension that took as long as 1 hr to be reversed. We then correlated the decline and recovery of tension with the instantaneous metabolic profile of the fiber. Prolonged tetanic stimulation reduced fiber phosphocreatine (PCr) in proportion to the internal work performed by the fiber, as measured by the isometric time-tension integral. However, despite the fact that ATP is the immediate energy source for muscle contraction, ATP concentration was normal or close to normal in fatigued fibers. ATP was not sequestered in internal compartments in these fibers, as residual ATP could be consumed when caffeine was applied to the fibers to produce maximal and irreversible contractures. Thus, fatigue was not due to depletion of available energy stores for muscle contraction, but arose from failure of coupling between excitation and contraction. On the basis of measured lactate and ATP/PCr ratios in fatigued fibers, we suggested that H^+, as lactic acid, accumulates during repetitive stimulation to interfere with calcium-activation of muscle contraction.

Significance to Biomedical Research and the Program at the Institute.

1. Defects in the properties of the blood-nerve barriers may contribute to peripheral neuropathies associated with disease and aging. Because little is known about the role of the perineurium in regulating the environment of peripheral nerves, it is important to experimentally obtain information about the tissue's basic properties.

2. Our finding that muscle fatigue develops despite high levels of ATP, corresponds to observation of "power failure" in stimulated peripheral nerve and sympathetic ganglia. In these tissues, function also fails before energy supplies are exhausted. Protection of energy supplies from exhaustion may preserve survival of neuronal and muscle cells. Muscle wasting and fatigue commonly accompany senescence in man, but may be partially reversed, as endurance training can reduce fatigue despite age (Suominen *et al.*, *J. Gerontol.* 32: 33, 1977). Endurance training also increases muscle aerobic metabolism. If our hypothesis is correct, that lactate accumulation rather than energy exhaustion contributes to fatigue, then the results in man may reflect an enhanced ability of endurance-trained muscles to oxidize lactic acid.

Proposed Course:

1. We will continue to investigate the function and morphology of the perineurium and its regulation of the peripheral nerve environment.

2. The data on muscle fatigue will be analyzed and prepared for publication.

Publications:

1. Weerasuriya, A., Rapoport, S.I., and Taylor, R.E.: Perineurial permeability increases during Wallerian degeneration. *Brain Res.* 192: 581-585, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG-00122-04 LNS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pharmacology of Central and Peripheral Catecholaminergic Nervous Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Resting Fischer-344 rats underwent a decrease in heart rate and an increase in diastolic blood pressure and plasma norepinephrine with increasing age. The heart rate and blood pressure responses of 28-month old rats were reduced as compared to 3-month old animals, although maximal norepinephrine release was unchanged.
2. Local glucose utilization (LGU) tends to increase in sympathetic ganglia between 12 and 24 months of age in the Fischer-344 rat. The increase is statistically significant in the superior cervical ganglia, and may be related to increased circulating norepinephrine.
3. Paraganglia, which contains catecholamines and are extra-adrenal chromaffin tissue, degenerate after birth in the rat but reappear between 24 and 33-months of age, and contain high concentrations of catecholamines.
4. Catecholamine fluorescence mapped from single neuron pericarya decreased with age in certain peripheral sympathetic ganglia as well as in certain brain regions which contain catecholamine-containing neurons.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

The objectives of this study are to (1) examine immobilization stress in unanesthetized rats in relation to aging, (2) to measure local glucose utilization (LGU) as an indicator of functional activity, in the sympathetic nervous system in relation to age, (3) to examine the morphology and role of paraganglia in relation to age, and to (4) measure catecholamine (CA) histofluoescence in individual neurons in peripheral sympathetic ganglia as well as in specific brain regions in relation to senescence. All studies were performed on the Fischer-344 rat.

Methods Employed:

1. A chronic indwelling tail arterial catheter was used to examine immobilized rats blood pressure of free-running and of stress. Analytical procedures were employed for the assay of plasma catecholamines (by radioenzymatic assay), corticosterone (by spectrofluorometric procedure) and plasma glucose (by enzymatic assay), corticosterone (by spectrofluorometric procedure) and plasma glucose (by enzymatic proceudre with Beckman glucose analyzer).

2. The method of formaldehyde-induced fluorescence (Eränkö, 1967) was used to demonstrate catecholamines at a cellular level with formaldehyde-induced fluorescence. It is based on the reaction of paraformaldehyde catecholamines, in which fluorescent isoquinoline derivatives of CA are formed (Exic. max 410 nm/emiss. max 480 nm). The method involves (1) freezing of tissue samples (in liquid nitrogen or in freon cooled with liquid nitrogen), (2) freeze-drying (10^{-5} torr; 7-10 days), (3) paraformaldehyde treatment (60 min., +80°C, 75% relative humidity) and (4) vacuum embedding into paraffin.

Quantitative microspectrofluorimetry. The intensity of formaldehyde-induced fluorescence is linear to CA-concentration. For recordings of fluorescence, MPV-2 Leitz microscope equipped with a photometer was used. The measuring spot was 10μ and allowed intracytoplasmic measurements from individual neurons. Fading of CA was prevented by an iris diaphragm on the excitation side.

^{14}C-2-deoxy-D-glucose technique. For determination of local glucose utilization in different peripheral sympathetic ganglia, the ^{14}C-deoxy-D-glucose technique was used as described by Sokoloff *et al*. (1977).

For electronmicroscopy, the body was rapidly perfused with 3% glutaldehyde in phosphate buffer (pH 7.3; 0.1 mol) through heart. The samples were later embedded in EPON, sectioned and studied with electron microscopy.

Major Findings:

1. In resting Fischer-344 heart rate declined and diastolic blood pressure and plasma norepinephrine increased with increasing age. The blood pressure of adult rats (3 and 12 months old) increased significantly during a 3-min immobilization (3 months to 24 months). The blood pressure of 24-month-old rats failed to increase during stress, decreased to below control immediately following the termination of stress. The aged animals died within 24 hours. Three minutes of

stress failed to increase the heart rate of old rats, but 30 min of stress produced a small increased heart rate, approximately 1/3 to 1/2 of the increase seen in 12 month-old animals. The old rats showed no decrease in maximal adrenergic medullary or cortical responses to stress, but they had a prominent 3 min delay in the maximal peak of norepinephrine release. Thirty min of immobilization induced a greater increase in plasma epinephrine in 24-month old rats than in 12-month old rats, and decreased beta-adrenergic responses such as heart rate and plasma glucose concentrations. The results indicate that old rats lose their ability to respond to and survive acute stress, due to a decreased adrenergic responsiveness and increased "shock" reaction (Ref. 1).

2. Paraganglia and Age. Paraganglia (PG) are located close to sympathetic ganglia. They contain cells which are classified as extra-adrenal chromaffin tissue, and contain catecholamines in high concentrations. Their physiological significance is uncertain. In the rat, extra-adrenal chromaffin tissue is abundant during fetal development, but degenerates postnatally. In the young adult rat, only a few small PG are found. Because the PG are a potential source of circulating catecholamines, the finding that plasma catecholamines are elevated in unstressed aged Fischer-344 rats (Ref. 1) led us to systematically study PG in peritoneal tissues of young and old Fischer-344 rats.

For identification of PG, samples of para-aortic tissue and of tissue containing the coeliac-mesenteric ganglion complex and the hypogastric ganglion were removed from 3- and 33-month old male Fischer-344 rats, and were processed by the formadehyde-induced fluorescence method for visualization of catecholamines. Small PG containing 5-30 cells per section were found consistently in young animals. In each of 6 old animals, however, large PG containing 500-4,000 brightly fluorescent cells per section were detected. Cell counting revealed a 13.5x increase in the number of PG cells between 3 and 33 months of age. Microspectrofluorimetric quantitation in the old rats showed equal amounts of catecholamines in PG cells and in adrenal medullary cells. Most PG were located in the para-aortic area.

3. Local glucose utilization (LGU) of the sympathetic nervous system and aging. LGU, as a measure of functional activity, was determined in the Fischer-344 rat in the superior cervical ganglion (SCG), the hypogastric ganglion (HGL) and the coeliac-mesenteric ganglion complex (CMGC) at 3, 12, 24 and 32-34 months of age. LGU increased significantly ($p < 0.05$) in the SCS between 12 and 24 months, and corresponded to an increase in circulating concentration of plasma norepinephrine in unstressed rats (Ref. 1). As heart rate and systolic blood pressure declined after 12 months of age (Ref. 1), the results suggest that there is increased functional activity, related to norepinephrine release, in the SCG with aging, as possible compensation for decreased responsitivity of peripheral receptors to circulating catecholamines. An abstract of this work has been published (Partanen, M., Rapoport, S.I., London E.D., and Hervonen, A. Glucose utilization in sympathetic ganglia of male Fischer 344 rats at different ages. Cellular and Molecular Mechanisms of Aging in Nervous Tissue. S. Vincent, Italy, August 31 - September 2, 1981).

4. Catecholamine neurons in peripheral and in central nervous system and aging. CA-histofluorescence was mapped in 3, 12, 24 and 32 month-old animals in peripheral sympathetic ganglia (SCG, HGL) and in the brain in epinephrine-containing neurons (A1-2), in norepinephrine neurons (A6-7) and in dopamine containing neurons (A_{8-10}).

In the brain, insignificant changes in CA-fluorescence were observed in A_6 and A_7 neurons. The only significant change, a decrease, was observed in A_{12} neurons which regulate pituitary hormones. This decrease occurred between 24 to 34 months. The changes may be related to hormonal patterns during aging. In peripheral sympathetic ganglia, CA-fluorescence decreased significantly in HGL. The changes might be related in changes in target organ, in neurons themselves or input to the ganglia. An abstract of this work has been published (Partanen, M., Santer, R., Rapoport, S.I., and Hervonenen, A. Microspectrofluorimetric quantitation of the age-related changes in the histochemically demonstrable catecholamines of central and peripheral neurons in the male Fischer- 344 rats. In: Cellular and Molecular Mechanisms of Aging in Nervous Tissue, St. Vincent, Italy, Aug. 31-Sept. 2, 1981).

Significance to Biomedical Research and the Program at the Institute.

An age-related study of the sympathetic nervous system, and or cardiovascular responsivity, in the Fischer-344 rat under resting and stressed conditions helps to elucidate the functional competence of these systems and to pinpoint defects which can be specifically examined, and related to human observations during stress. The increased sympathetic activity with age in the unstressed rat, as measured by increased plasma catecholamines and reappearance of the paraganglia, may be related to decreased responsivity of the cardiovascular and other systems to catecholamines. Thus the studies suggest a feed-back between end organ effect and sympathetic activity that is age-dependent. Because many of the changes in rats are similar to those in man (plasma catecholamines increase in man with age, and paraganglia reappear in human peritoneal tissue), the rat may be a good model for studying regulation of the cardiovascular system by the sympathetic nervous system in aged man.

Proposed Course: To get morphological and mapping studies with age in sympathetic ganglia and in PG paraganglia. To study catecholamine turnover by combined drug treatment and quantitative microspectrofluorimetry in sympathetic ganglia, as well as in PG. To study what regulates the appearance of PG in old animals. To study the regulation of catecholamines from PG-cells.

Publications:

1. C.C. Chiueh, S.M. Nespor and S.I. Rapoport. Cardiovascular, sympathetic and adrenal cortical responsiveness of aged Fischer-344 rats to stress. Neurobiology of Aging 1, 157-163, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00123-03 LNS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Synapse Development, Specificity and Mechanism in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Thompson	Sr Staff Fellow	LNS	NIA
Others:	S.I. Rapoport	Chief	LNS	NIA
	C.L. Makino	Biochem. Lab Tech	LNS	NIA
	J.E. Johnson, Jr.	Guest Worker	OSD	NIA
	B.A. Suarez-Isla	Visiting Fellow	LNS	NIA

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Section on Experimental Morphology, OSD, NIA

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Synapse formation, synapse specificity and neurotransmission were studied using neurons and muscle cells in culture. Synapses were detected and investigated by electrophysiological recording. Neurons from chick and rat retina and chick spinal cord form synapses with muscle cells. Each type of neuron possesses a synapse-competent state in its development during which it can abundantly form synapses with muscle cells in culture. In addition, developmental periods for maximal neurite extension correlate with the developmental periods for synaptogenesis. These results suggest a mechanism of synapse specificity based on a synaptogenic period during development for each type of neuron.

PHS-6040
(Rev. 2-81)

Project Description

Objectives: Neurons in culture form synapses with other neurons and/or muscle as shown by electrophysiological recording and electron microscopy. There is a discrete developmental period for synapse formation which may be specific to the type of neuron. Older neurons appear to lose their ability to form synapses or may only form relatively few synapses whereas neurons from younger animals have a greater capacity to form synapses. The objective of this study is to examine the properties that determine specific synapse formation during development in relation to neuronal type and target organ. In addition, the objective is to examine which parameters determine whether a synapse is appropriate and is retained or whether a synapse is inappropriate and is lost during further development. In examining these questions, neurons and their target cells are established in a culture environment which can be manipulated in order to study a) neurotransmission, b) synapse formation, c) synapse termination and d) synapse specificity. This system allows one to study how development, aging, drugs and disease affect the above properties of neuronal connections.

Methods Employed: Striated muscle cells isolated from newborn rats grow in culture, form myotubes, develop nicotinic acetylcholine receptors and become electrically active. Neurons from chick, mouse or rat spinal cord and retina, and from neuroblastoma cell lines form synapses with these muscle cells in culture. Synapses are detected by electrophysiological recording of miniature endplate potentials with microelectrodes in target muscle cells. Biochemical measurements are made of neurotransmitter levels, release and synthetic enzymes. In addition, morphological measurements at the light and ultrastructural levels are made on the cultured cells.

Major Findings: 1) Synapse development. The ability of neurons at different development ages to form synapses in culture was studied by combining neurons from chick embryos with rat striated muscle cells as target organs. Synaptic connections were examined after 24 hr of co-culture by looking for miniature endplate potentials in muscle cells with microelectrodes. Three populations of cholinergic neurons from the chick embryo were examined. Previous studies (Puro, D.G., DeMello, F.G. and Nirenberg, M., Proc. Natl. Acad. Sci., USA, 74: 4977-4981, 1977; Ruffolo, R.R., Jr., Eisenbarth, G.S., Thompson, J.M. and Nirenberg, M., Proc. Natl. Acad. Sci., USA, 75: 2281-2285, 1978) have shown that chick embryo retinal neurons form synapses on rat striated muscle cells in culture only during a discrete period in their development. Synapses are only formed by neurons taken from chick embryos between the ages of 6- and 16-days *in ovo*, with 8-day neurons forming the greatest number of synapses. In further studies, as yet unpublished (Thompson, J.M., Ruffolo, R.R., Jr., Eisenbarth, G.S. and Nirenberg, M.), it was shown that rat retinal neurons formed synapses only between the 18th day of embryonic development and day 7 after birth, with a maximum number formed from pups 1 day old. Neurons from the chick embryo spinal cord formed synapses after 2 days, the maximum number with 4-day neurons. The capacity to form neurons decreased with age, and was observed after 51 hr, but not after 24 hr, of co-culture with neurons from 18-day embryo spinal cord. Thus, spinal cord neurons do not completely lose their ability to form synapses with muscle cells. All neurons tested show decreased ability to form synapses as neurons age during development. The neurons also lose their ability to form synapses when maturing *in vitro* in a manner and time course which parallels the loss of their ability to form synapses during *in ovo* maturation.

Neurite extension by neurons in vitro also changes with development. There is a statistically significant age-related decrease in the percentage and average length of neurites formed in 24-hr cultures of chick retina and spinal cord neurons between 6- and 16-days of embryonic age (J.M. Thompson, Developmental Decrease in Neurite Extension in Cultured Chick Embryo Retina and Spinal Cord Neurons. Soc. Neurosci. Abstr. 6: 327, 1980). These results imply that maximal rates of neurite extension and synaptogenesis occur concurrently during development and, thus, suggest that neurite extension is critical to synaptogenesis. It remains to be determined whether the maximal rates are casually related, and whether the developmental periods correlate in ovo as they do in vitro.

It has been proposed (Ruffolo, R.R., Jr., Eisenbarth, G.S., Thompson, J.M. and Nirenberg, M., Proc. Natl. Acad. Sci. USA. 75: 2281-2285, 1978) that sequence of neuronal birth, the relative location of the neurons within the organ, the developmental stage of neurons, and the length of their synapse competent state may restrict synapse targets to cells which have reached a developmental period in which they are receptive to forming synapses. Thus, specific neuronal connections can be formed. Synapse plasticity and/or regeneration in older animals may be restricted due to these developmental changes.

The use of primary neuronal cultures is limited by the fact that differentiated neurons do not divide in culture. Neuroblastoma cell lines, neuronal cancers which can replicate in culture, have become important in vitro model neuronal systems. In addition, they can be used as models of in vitro aging. However, few of the commonly available cell lines have been systematically compared in terms of their ultrastructure, function and neurochemistry. Preliminary results (Johnson, J.E., Jr., and Thompson, J.M. Cancer Cells as a Model for Studies of Neuronal Aging, The Gerontologist, 20: 133, 1980) have shown that three commercially available neuroblastoma cell lines are not all homogeneous as determined by scanning electron microscopy suggesting that more than one cell type is present in two of the three cell lines. Further studies will compare the ability of each cell line to form synapses with muscle cells in vitro and the levels of several neurotransmitter synthetic enzymes.

Significance to Biomedical Research and the Program at the Institute:

Irreversible alteration of neuronal properties during development limits the capabilities of mature and senescent neurons. Since neurons can readily form synapses only during a discrete period in their development, the number of new synapses which older neurons can form may be limited. Thus, synapse plasticity and regeneration which may be important in learning and memory or recovery from disease or trauma could be limited in older organisms.

The determination of properties of neuroblastoma cell lines will allow the appropriate selection of cells for studies of neuronal aging, synapse formation and neurotransmission.

Proposed Course: Neuronal and neuronal-muscle co-cultures will be used to study: (a) pharmacology of synapses and synaptic circuits, (b) pharmacology of neurotransmitter release and changes during development and aging, (c) comparison of neuroblastoma cell lines as model systems for study of neuronal properties, (d) control of synapse-competent states of neurons, and (3) regulation of synapse stabilization and termination as a mechanism of synapse specificity.

Publications:

Thompson, J.: Critical Periods for Synapse Formation: Implications for Plasticity. In Adelman, R.C., Baker, G., Cristofalo, V. and Roberts, J. (Eds.): Modern Aging Research, 1, New York, Alan R. Liss, 1980, pp. 221-222.

Nirenberg, M., Wilson, S., Higashida, H., Thompson, J., Eisenbarth, G., Walsh, F., Rotter, A., Kenimer, J., and Sabol, S.: Synapse plasticity. Pontificiae Academiae Scientiarum Scripta Varia, 45: 123-127, 1980.

Thompson, J.M., and Rapoport, S.I.: Developmental decrease in neurite extension in cultured chick embryo retina and spinal cord neurons. Devel. Biol. 84: 244-246, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00124-03 LNS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Central Nervous System Binding Sites for the Neurotoxin Kainic Acid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	H.I. Yamamura	Professor	Univ of Arizona

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Department of Pharmacology, Univ of Arizona Hlth Science Center, Tucson, AZ.

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Gerontology Research Center, Laboratory of Neurosciences

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The specific binding of $[^3H]$kainic acid (KA) to high- and low-affinity receptor sites was assayed in postmortem samples from the brains of patients affected with Huntington's disease (HD) and age-matched controls. Treatment of rat brain with conditions that closely mimic the temperature gradient occuring in postmortem human brain did not significantly decrease receptor binding 12 hr after death. In HD brains, total specific binding of $[^3H]$KA was reduced in the caudate nucleus by 51%, putamen by 77%, and frontal cortex by 47%. Specific binding to the high-affinity site was virtually undetectable in the caudate nucleus and was reduced by 90% in the putamen from HD brains. No significant alterations in specific binding of $[^3H]$KA were noted in the insular or temporal cortex, hippocampus, or cerebellum. Thus, losses of KA receptor binding were mainly localized to those regions of the HD brain that are most severely affected by neuronal degeneration, and the high-affinity receptor site appeared more affected.

PHS-6040
(Rev. 2-81)

Project Desciption.

Objectives: Intrastriatal injection of the neurotoxin kainic acid (KA) to rats has provided an animal model for the human neurodegenerative disorder Huntington's disease (HD). Subtle modifications in the structure of KA eliminate its neurotoxic effects, indicating a high selectivity in the recognition sites mediating kainate's action. The marked potency of KA as a depolarizing agent indicates that these sites have a high affinity for the molecule. Kinetic analysis of the specific binding of $[^3H]$KA to membranes isolated from the human brain have revealed both high affinity and lower affinity sites similar to those characterized in rat and other species. In the present study, the specific binding of $[^3H]$kainic acid to high and lower affinity sites was measured in various regions of postmortem brain obtained from patients dying with HD and from age-matched controls.

Methods Employed:

1. Preparation of washed membranes. Tissue for assay is taken from hydra, sea anemone, planaria, earthworm, crayfish, cockroach, goldfish, frog, hagfish, spiny dogfish, chick, rat and human postmortem brain. Samples are sonified in glass distilled water, and the suspensions centrifuged at 48,000 x g for 10 min at 2°. The membrane pellets are resuspended, pelleted and washed sequentially in 100 volumes of glass distilled water and 50 mM Tris-citrate buffer, pH 7.1. Finally, the membranes are suspended in 100 vol of 100 mM Tris-citrate buffer, pH 7.1. Protein is assayed by the method of Lowry *et al*., 1951.

2. Standard $[^3H]$kainic acid binding assay. 1 ml of washed membrane suspension and various concentrations of radioligand displacers are placed in 15 ml Sorvall teflon centrifuge tubes. Ten to 300 pmoles of freshly diluted $[^3H]$KA are added in a volume of 100 µl to start the reaction. The incubation volume is adjusted to 2 ml with distilled water. Incubations are run for 60 min at 2° and are stopped by centrifugation. Bound radioactivity in pellets is estimated by liquid scintillation spectroscopy. Nonspecific binding is determined in the presence of 0.1 mM unlabeled KA.

To characterize separately binding to high and low affinity sites, advantage is taken of the fact that the radioligand dissociates slowly from high affinity sites but nearly instantaneously from low affinity sites in rat and human brain. After 60 min of incubation with $[^3H]$KA, 200 nmoles of unlabeled KA are added to some tubes, bringing the final concentration to 0.1 mM. After another 5 min of incubation, the samples are centrifuged and bound radioactivity measured. The addition of unlabeled ligand 5 min prior to centrifugation displaces radioligand from low affinity binding sites but does not affect nonspecific or high affinity binding.

Major Findings:

1. Postmortem changes: Conditions that closely mimic the core temperature gradient in brains of human cadavers for a period up to 12 hr did not significantly affect total specific binding in rat brain. In addition, the amount of binding to high-affinity and lower affinity receptor sites did not differ

significantly in striatum frozen immediately after decapitation as compared to binding in striata subjected to the gradual cooling. Thus, the receptor-binding sites for kainic acid appear to be relatively stable under conditions typical for human postmortem brain material.

2. Specific binding of [^{3}H]kainic acid in Huntington's diseased and control brain. Total specific binding of [^{3}H]kainic acid in the caudate nucleus was reduced by 51%; high-affinity binding for the HD samples was not significantly above blank and in eight of ten samples was undetectable. Similarly, total specific binding in the putamen was reduced by 77% with a 90% reduction in high-affinity binding in HD brain. Although in the globus pallidus (GP) there were no significant effects on the mean levels of specific binding, in four of the seven GP samples from HD brains, specific binding was undetectable; in contrast, all GP samples from controls had measurable, albeit low, levels of specific binding. In the frontal cortex, total specific binding in HD samples was reduced by 47% with no significant alteration in the high-affinity site. No significant differences were observed between HD and control samples of insular cortex, temporal cortex, hippocampus, or cerebellum.

Significance to Biomedical Research and to the Program of the Institute.

These studies demonstrate that KA receptor sites are significantly reduced in brain regions affected by neuronal degeneration in HD. Significant reduction in the total specific binding of [^{3}H]KA was observed in the caudate, putamen, and frontal cortex of HD patients. In both the caudate and putamen, the high-affinity sites were profoundly affected in HD. Notably, brain areas not severely involved in the degenerative process of HD such as insular cortex, hippocampus, and cerebellum, did not exhibit significant alterations in kainic acid receptor binding.

One must question whether the loss in KA receptors is intrinsically involved in HD or is merely a secondary phenomenon associated with neuronal degneration. Previous studies of other neurotransmitter receptors in HD have revealed losses primarily in the caudate and putamen; and in the cases of dopamine, muscarinic acetylcholine, and GABA receptors, the reduction has been in the range of 40-60%. Thus, it is noteworthy that the high-affinity KA receptor site is more profoundly affected in the caudate and putamen than these other receptors. These results lend support to the hypothesis that an altered disposition of an endogenous substance that interacts with KA receptors may be responsible for the neuronal degeneration in HD. However, alternative hypotheses concerning a cellular membrane defect that renders neurons uniquely vulnerable to glutamate-induced depolarization in a manner analogous to the exogenous kainate challenge cannot be ruled out.

Recently, local injections of KA have been used to selectively reduce the cholinergic innervation of the cortex in rats, resembling the major reported neurochemical deficit in senile dementia of the Alzheimer type. Thus, the information obtained in these studies seem relevant not only to HD, but to several human diseases where specific cell loss may be linked with a membrane receptor-mediated phenomenon.

Proposed Course: Project discontinued.

Publications:

1. London, E.D., Klemm, N.,, and Coyle, J.T. (1980): Phylogenetic distribution of [^{3}H]kainic acid receptor binding sites in neuronal tissue. *Brain Res.* *192*: 463-476.
2. London, E.D., Yamamura, H.I., Bird, E.D., and Coyle, J.T. (1981): Decreased receptor binding sites for kainic acid in brain of patients with Huntington's disease. *Biol. Psychiatry*, *16*, 155-162.
3. Coyle, J.T., Slevin, J., London, E.D., Biziere, K., and Collins, J. (1981) Characterization of neuronal recognition sites for [^{3}H]kainic acid. In *Psychopharmacology and Biochemistry of Neurotransmitter Receptors*, ed. by R.W. Olsen, H.I. Yamamura and E. Usdin. Raven Press, New York, pp. 501-514.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00125-03 LNS

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cerebral Metabolism, Relation to Brain Function and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.D. London	Sr Staff Fellow	LNS	NIA
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TOTAL MANYEARS: 6.7 | PROFESSIONAL: 4.1 | OTHER: 2.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) Cerebral metabolism was studied in relation to age and drug responses in unanesthetized animals. Local cerebral glucose utilization (LCGU), as measured with ^{14}C-2-deoxy-D-glucose, increased during brain growth in the Fischer-344 rat, and declined during adulthood in the Fischer-344 rat until midlife, without subsequent decrements. In the beagle, LCGU decreased progressively throughout the lifespan, but most of the significant decrements had occurred by mid-life. In the Fischer-344 rat, regional cerebral blood flow (rCBF), measured with ^{14}C-iodoantipyrine, was not elevated by immobilization. rCBF rose during development and declined in some regions after 12 months of age. However, the cerebral metabolic rates for O_2, glucose and CO_2, which reflect cerebral cortical oxidative metabolism, did not decline significantly between 3 and 24 months of age. 2) As retinal degeneration progressed with age and light exposure in the albino rat, a loss of photoreceptor cells was correlated with a decline in retinal glucose utilization. 3) In adult rats, central muscarinic stimulation with oxotremorine increased LCGU in areas involved in motor and cognitive function. GABA agonists generally decreased LCGU, but produced a relative increase in the dentato-rubro-thalamic pathway. 4) Fatty acid uptake by the brain was examined quantitatively.

PHS-6040
(Rev. 2-81)

Objectives: The aims of this program are: To examine morphological, biochemical and functional changes in the brain that occur during development, maturation and aging; to study coupling between regional cerebral blood flow and metabolism; to examine cerebral metabolism in response to specific centrally-acting drugs. Specifically, the program is designed to (1) describe the time course of changes in local cerebral glucose utilization (LCGU) during development and aging of the unanesthetized Fischer-344 rat and beagle, (2) to measure regional cerebral blood flow (rCBF) during development and aging of the rat, (3) to relate retinal morphology to retinal glucose utilization during aging of the rat, (4) to study global cerebral blood flow (CBF) and the cerebral metabolic rates for O_2 ($CMRO_2$), glucose (CMRglc) and CO_2 ($CMRCO_2$) in aging Fischer-344 rats, (5) to study the effects of muscarinic and of (6) GABAergic drugs on LCGU, (7) to study the effect of immobilization stress on rCBF in the adult rat and (8) to examine fatty acid incorporation by the rat brain.

Methods Employed: 1. For studies of LCGU in rats and beagles, experiments are performed on partially restrained, unanesthetized animals with indwelling femoral arterial and venous catheters. ^{14}C-2-deoxy-D-glucose (^{14}C-2-DG) (125 µCi/kg in rats; 50 µCi/kg in beagles) is injected i.v., and timed arterial blood samples are collected over the next 45-50 min until the animal is killed. Regional brain radioactivity is determined by dissection and liquid scintillation spectroscopy, or by quantitative autoradiography on 20µ frozen sections. LCGU is calculated from the tissue radioactivity at kill time, the plasma histories of glucose and ^{14}C-2-DG, and the kinetic constants for ^{14}C-2-DG uptake and metabolism by brain (Sokoloff et al., 1977, J. Neurochem. 28: 897). For the rat, a lumped constant of 0.483 (Sokoloff et al., 1977, J. Neurochem. 28: 897) is used. The lumped constant used for the beagle is 0.558 (Duffy et al., Trans. Am. Soc. Neurochem. 10: 171, 1979).

2. Retinal glucose utilization (RGU) is measured as is LCGU (see above) on light-adapted retinas removed from rats administered ^{14}C-2-DG. Morphological observations are made by light microscopy on plastic-embedded 1 µ-sections of retina strips.

3 & 7. For studies of rCBF, conscious restrained rats, prepared as described above, are infused i.v. with ^{14}C-iodoantipyrine and decapitated 45-55 sec later. Regional brain radioactivity and whole blood radioactivity are determined by liquid scintillation spectroscopy. rCBF is calculated by applying the Kety-Schmidt analysis to the data (Sakurada et al., 1979, Am. J. Physiol. 234: H59; Ohno et al., 1979, Stroke 10: 62). Arterial blood pH, $PaCO_2$ and PaO_2 are measured with specific electrodes.

4. The cerebral metabolic rates for O_2, glucose and CO_2 are obtained by placing an indwelling needle in the superior sagittal sinus of a conscious rat, and measuring the concentrations of these substances in sagittal sinus blood and femoral arterial blood. The arterio-venous differences are multiplied by CBF, as determined with ^{14}C-iodoantipyrine.

5. The effects of central muscarinic stimulation on LCGU are assessed in adult rats treated with oxotremorine (0.75 mg/kg, i.p.) 2 min before i.v. ^{14}C-2-DG. Atropine methylbromide (1 mg/kg, s.c. 20 min before oxotremorine) is given to block peripheral muscarinic effects. Scopolamine (2.5 mg/kg, i.p.) is given 10 min before oxotremorine to test the specificity of the effect of oxotremorine on central muscarinic receptors.

6. The effects of GABAergic drugs on LCGU are assessed in adult rats injected i.v. with one of several doses of the GABAergic agonist (muscimol, 1-7 mg/kg; 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol, THIP, 12 and 24 mg/kg) or antagonist (bicuculline, 0.3 mg/kg) prior to ^{14}C-DG.

8. ^{14}C-palmitate is injected i.v. in conscious rats, and incorporation of tracer is measured in brain regions in relation to plasma concentrations.

Major Findings

1. Cerebral and Retinal Glucose Utilization and Cerebral Blood Flow in Relation to Aging.

a. Age-related changes in LCGU in the Fischer-344 rat and the beagle. Between 1 and 3 months of age, glucose utilization tended to increase in all brain regions; statistically significant increases occurred in seven regions. Between the ages of 3 and 12 months, glucose utilization decreased significantly in 12 regions. The greatest reductions (25% or more) occurred in the striatum, inferior colliculus, and pons, but the hypothalamus and thalamus, nucleus accumbens, and septum showed no statistically significant change. LCGU did not change between 12 and 24 months or between 24 and 34 months of age. The results demonstrate a rise in cerebral glucose utilization with development from 1 to 3 months, a decline between 3 and 12 months, and a constancy in the second and third years that does not reflect reported senescence-associated neurochemical and morphological cerebral changes. These results have been published in abstract form (London, E.D., Ohata, M. and Rapoport, S.I. (1980): Local cerebral glucose utilization and regional blood flow during development, maturation and aging of the Fischer-344 rat. The Gerontologist 20, No. 5, part II, 252)(Ref. 1).

LCGU was measured in 24 brain regions of 1, 3, 6, 10-12 and 15 year-old beagles. As in the rat brain, most age-related decrements occurred by mid-life. In most brain regions, LCGU declined progressively from one to six years, with no significant changes thereafter. The only regions which showed significant decrements after six years were the mamillary bodies and geniculate bodies. A preliminary report of these results has been published (Garden, A.S., Ohata, M., Rapoport, S.I. and London, E.D. (1980): Age-associated decrease in local cerebral glucose utilization (LCGU) in the beagle. Abstracts Tenth Annu. Meeting Soc. Neurosicence 6: 768).

b. Age-related changes in the retina of the Fischer-344 rat. Progressive degeneration was observed throughout the retina with age, but was most rapid and severe in the periphery. The central retina was more severely affected than were the equatorial regions. Glucose utilization in light-adapted retinas did not change between 1 to 3 months, although there was significant cell loss in the photoreceptor and inner nuclear layers. From 3 to 34 months, there was a significant decrease (76%) in glucose utilization which was associated with an 86% decline in the number of photoreceptor nuclei at the peripheral, equatorial and central retinal regions. The number of inner layer nuclei decreased slightly during this period. Abstracts of this work have been published (a) Shinowara, N.L., Rapoport, S.I., Hoover, M.J. and London, E.D. (1980). Comparison of retinal changes in morphology and glucose utilization in Aging Fischer-344 rats. In: Neural Regulatory Mechanisms During Aging. Proc. Third Phil. Symp. on Aging. Alan R. Liss, Inc., New York (Abstr.). (b) Shinowara, N.L., Rapoport, S.I., Hoover, M.J. and London, E.D. (1980). Changes in retinal morphology and local glucose utilization (LCGU) in 1 to 34 month old Fischer-344 rats. Neurosci. Abstr. 6: 346.

c. Age-related changes in regional cerebral blood flow (rCBF) between 1 and 34 months in the Fischer-344 rat. In 1 mo old unanesthetized rats, mean rCBF in 9 anterior brain regions was 92 ml.100 g^{-1}.min^{-1} and was 76 ml.100 $g^{-1}$$min.^{-1}$ in 4 posterior brain regions. rCBF increased significantly ($p < 0.05$) in the anterior regions between 1 and 3 mo of age, and continued to increase after 3 mo to a mean of 132 ml.100 g^{-1}.min^{-1} at 12 mo, but did not change significantly in the posterior brain regions between 1 and 12 mo. rCBF tended to decline between 12 and 24 months, more consistently in the posterior than anterior brain regions. White matter rCBF did not change with age. rCBF did not follow the same temporal pattern during maturation and aging of the rat brain as did LCGU (see above). Separate time courses suggest age-related changes in the sensitivity of the cerebrovascular bed to metabolic factors which regulate rCBF (Ref. 2).

d. Cerebral blood flow and cerebral metabolic rates for oxygen, glucose and carbon dioxide in Fischer-344 rats of different ages. The cerebral metabolic rates for O_2 ($CMRO_2$), glucose (CMRglc) and CO_2 ($CMRCO_2$), were calculated from the concentrations of these substances in femoral arterial and sagittal sinus blood of partially-immobilized unanesthetized Fischer-344 rats, at the ages of 3, 12 and 24 months. The arterio-venous differences were multiplied by cerebral blood flow (CBF). In fourteen 3-month old rats, CBF = 1.20 ± 0.08 ml/g/min (mean ± S.E.M.), $CMRO_2$ = 4.71 ± 0.32 μmol/g/min, CMRglc = 0.72 ± 0.09 μmol/g/min, and $CMRCO_2$ = -54.86 ± 1.17 μmol/g/min. Whereas CBF, $CMRO_2$ and $CMRCO_2$ did not change significantly with age ($p > 0.05$), CMRglc was greater at 12 mo than at 3 mo. The ratio $CMRO_2$/ CMRglc was 6.97 ± 0.51 at 3 mo, and also did not change significantly with age. In individual rats, CBF was positively correlated with $CMRO_2$ and CMRglc ($p < 0.05$), due to coupling between blood flow and oxidative metabolism, and negatively correlated with arterial hematocrit (which was reduced by blood loss). As the superior sagittal sinus drains venous blood mainly from supratentorial cortical regions, the results indicate that global CBF and indices of cerebral cortical oxidative metabolism do not decline with age in the conscious partially-immobilized rat. The results are not in conflict with previous observations in aging rats, that decreases in glucose utilization in most cortical areas are usually less than 20% (Ref. London _et al_., _J. Neurochem_., in press). Such small decrements may be obscured by experimental errors in determinations of global CBF, CMRglc and regional CMRglc. An abstract of this work has been published (Takei, H., Fredericks, W.R., London, E.D. and Rapoport, S.I. Cerebral blood flow and cerebral metabolic rates for oxygen, glucose and carbon dioxide in Fischer-344 rats of different ages. _Abstr. Eleventh Soc. Neurosciences_, 1981).

2. _Cerebral Glucose Utilization in Response to Drugs_.

a. _Effects of a muscarinic cholinergic agonist on cerebral metabolism in the adult rat_. Systemic administration of the muscarinic agonist oxotremorine (OXO, 0.7 mg/kg, i.p.) increased LCGU in brain regions involved in motor function: the sensorimotor cortex; the extrapyramidal motor system, including the striatum, globus pallidus, red nucleus, substantia nigra, subthalamus and ventral nucleus of the thalamus; the cerebellar vermis, fastigial nucleus and nucleus interpositus; and the vestibular nucleus. No effects were observed in the fibers of the pyramidal tract, internal capsule, cerebellar white matter, and the dentate nucleus. Increases were not affected by methylatropine (1 mg/kg s.c.), but were completely antagonized by scopolamine (2.5 mg/kg, i.p.).

OXO also enhanced LCGU in brain regions which form the Papez circuit and are important in cognitive function (anterior nucleus of the thalamus, mamillary body, retrosplenial cerebral cortex, subicular areas). Other portions of the limbic system, such as the CA_1 and CA_3 regions of the hippocampus and the dentate gyrus, did not have altered LCGU. These results have been published in abstract form (Dam, M., Holloway, H., Mahone, P., Dow-Edwards, D. and London, E. (1981): Oxotremorine increases glucose utilization in the Papez circuit. _Trans. Am. Soc. Neurochem._, _12_: 220).

b. _Effect of GABAergic drugs on cerebral metabolism in the Fischer-344 rat_. GABA agonists induced dose-dependent depressions of LCGU in most brain regions. Decreases were most dramatic in forebrain regions, especially the cerebral cortex, caudate nucleus and thalamic nuclei. There were variable reductions in LCGU within the hippocampus. In contrast, LCGU in the red nucleus was increased by up to 100% with a high dose of muscimol (7 mg/kg). Components of the dentato-rubro-thalamic pathway showed a relatively enhanced LCGU compared with the rest of the brain. Bicuculline (0.3 mg/kg) produced convulsions and increased LCGU in several regions, notably the auditory cortex and inferior colliculus. Muscimol (4 mg/kg) blocked the convulsions and increased LCGU. The LCGU responses to the GABA agonists differed from the response to pentobarbital, which produced an overall depression in LCGU

3. Immobilization and Cerebral Blood Flow

a. _Effect of immobilization stress on regional cerebral blood flow in the conscious rat_. Stress and anxiety have been reported to increase overall and regional cerebral blood flow (rCBF) in conscious man (Kety, 1950; Ingvar, 1978). We thought it important to evaluate its effect on conscious rats, in which we are studying both rCBF and LCGU under conditions of partial immobilization, by examining whether there is an effect of more complete immobilization. Recently, Carlsson et al. (Acta Physiol. Scand. 95: 206-208, 1975) reported that immobilization can increase brain O_2 consumption and CBF by as much as 100%.

Immobilization of conscious, normotensive, freely breathing 10-month-old Wistar-Kyoto rats produced an overall decline in rCBF, as measured with ^{14}C-iodoantipyrine, except at the frontal lobe. In 14 brain regions rCBF fell by an average of 14.3% after 5 min of immobilization and by 11.9% after 15 min. Immobilization also stimulated hyperventilation and thereby reduced $PaCO_2$. The slope relating rCBF to $PaCO_2$ averaged 1.5 ml/10 g/mm Hg in 9 significantly affected regions. The findings suggest that rCBF declines during immobilization stress because of cerebrovascular constriction caused by a reduction in $PaCO_2$. Furthermore, comparison of the average slope with published values indicates that if $PaCO_2$ remained unchanged during immobilization, rCBF would increase by at most 20%. The results do not confirm the findings of a marked stress effect, as reported by Carlsson et al. (1976). An abstract of this work has been published (Rapoport, S.I., Ohata, M., Sundaram, U. and Fredericks, W.R. 1981. Regional cerebral blood flow during immobilization stress in the conscious rat. J. Cerebral Blood Flow Metab. 1 (Suppl. 1): S477-S478.

b. Regional fatty acid uptake by rat brain. Small amounts of fatty acids are normally taken up from blood and incorporated into brain lipids. It was of interest to study the uptake and regional distribution of a saturated fatty acid, palmitic acid, in brains of conscious rats. Osborn-Mendel rats (250-350 g) were anesthetized with pentobarbital, after which femoral venous and arterial catheters were implanted. The rats were allowed to regain consciousness, ^{14}C-palmitate was administered i.v., and plasma and brain radioactivities were followed for up to 24 hr. Plasma radioactivity was 100% palmitate for the first 30 min, after which increasing amounts of labeled phospholipids and triglycerides appeared. Brain radioactivity increased in the first 20 min after injection, remained constant for 40 min, and then decreased by 50% at 2 hr, after which it remained unchanged for 22 hr. White matter accumulated and retained lower quantities of radioactivity than did gray. The inferior colliculus, olfactory tubercle, cerebellar gray and cortical areas accumulated and retained consistently higher radioactivity than did other gray matter regions. ^{14}C-palmitate, administered under the given experimental conditions, might be used to assess the relation between regional uptake and function of the brain. An abstract of this work has been published (Kimes, A.S. and Rapoport, S.I. Regional fatty acid uptake into rat brain. Abstr. Eleventh Annu. Meeting Soc. Neurosci. 7, 1981).

Significance to Biomedical Research and the Program at the Institute.

1. A knowledge of the regional metabolic deficits that appear during aging can direct future research to identify particular neurotransmitter systems which may be inadequate in elderly man. Our observations are that resting LCGU does not decline with senescence of the rat and suggests that pharmacological or physiological challenges are required to demonstrate functional deficits.

2. Retinal degeneration is influenced by age, light and disease. Our study, which correlates glucose utilization in the light-adapted albino rat retina with morphological changes involved in retinal degeneration, represents a way to examine functional deficits in relation to cell loss.

3. Cerebral functional activity correlates with rCBF and LCGU. The different time courses of rCBF and LCGU, during development and aging of the rat brain, suggest that rCBF and LCGU are not identical indices of funtion, and that both must be examined to interpret aging changes in the brain.

4. Measurements of $CMRO_2$, CMRglc, $CMRCO_2$ and CBF represent direct ways to evaluate oxidative metabolism of the cerebral cortex during aging. The invariance of these parameters in the brain of the rat during aging suggests that functional activity does not decline in the cortex, despite many age-related neurochemical and morphological changes.

5. Local cerebral metabolic responses to stimulation by the muscarinic agonist oxotremorine are relevant to the pharmacology of aging and senile dementia of the Alzheimer type. In view of presynaptic muscarinic loss in man in aging and to a greater extent in dementia, treatment has been designed to enhance central muscarinic neurotransmission. More importantly, our findings suggest that cholinergic agonists may enhance cognitive function by interacting with components of the Papez circuit, but also can produce untoward dose-dependent extrapyramidal stimulation.

6. The relative increase in LCGU in components of the dentato-rubro-thalamic pathway in response to GABAergic drugs, may explain the paradoxical "epileptic" action of GABA agonists. Furthermore, in view of the known interaction between GABAergic systems and benzodiazepines, knowledge of the distribution of the metabolic responses to GABA agonists could help explain untoward central effects of benzodiazepines in the elderly.

7. The examination of cerebral functional activity during stress, via the measurement of rCBF, is critical to understanding the value of baseline measurements of glucose utilization and rCBF in animals and man, under apparently resting conditions.

8. Fatty acid incorporation by brain may prove useful in the study of brain changes with aging and pathology.

Proposed Course: Data on age-related changes in LCGU, rCBF, retinal morphology and retinal glucose utilization, $CMRO_2$, CMRglc and $CMRCO_2$ will be analyzed and prepared for publication. The effects of cholinergic and GABAergic agonists on LCGU in the rat will be further investigated, and related to age. Further work will be performed on fatty acid incorporation by brain.

Publications:

1. Smith, C.B., Goochee, C., Rapoport, S.I. and Sokoloff, L. Effects of ageing on local rates of cerebral glucose utilization in the rat. Brain 103, 351-365, 1980.

2. Ohata, M., Sundaram, U., Fredericks, W.R., London, E.D. and Rapoport, S.I. Regional cerebral blood flow during development and aging of the rat brain. Brain 104, 319-332, 1981.

3. Palacios, J.M., Kuhar, M.J., Rapoport, S.I. and London, E.D. Increases and decreases in local cerebral glucose utilization in response to GABA agonists. Eur. J. Pharmacol. 71, 333-336, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00126-02 LNS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Brain Function in Aging and Disease in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.4	3.1	1.3

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Regional cerebral glucose utilization was measured, using positron emission tomography, in normal male volunteers at different ages. ^{18}F-2-deoxy-D-glucose was injected intravenously, and brain radioactivity was scanned at different times thereafter. Subjects were screened for the absence of cardiovascular disease, or of secondary or primary brain disease. One normal volunteer was found in whom glucose utilization as well as regional cerebral blood flow, were reduced in the right parietal lobe in the absence of signs or symptoms. In addition, protocols were written for the application of positron emission tomography to subjects with Organic Dementia of the Alzheimer Type.

Project Description

Objectives: The aim of this project is to relate behavioral and cognitive performance in normal man at different ages to the regional cerebral metabolic rate for glucose ($rCMR_{glu}$), as a measure of regional cerebral functional activity. The findings in normal man will be compared to findings in subjects with organic dementia of the Alzheimer type and with a history of infantile autism.

Methods Employed: Male subjects evenly distributed across the adult age spectrum are screened by medical diagnostic procedures for the absence of neurologic and cardiorespiratory disease, including arteriosclerosis. Personality and psychometric tests and a comprehensive neuropsychological battery are administered to evaluate cognitive performance. Electroencephalography and computerized axial tomography are employed to evaluate neurological status. Positron Emission Tomography (PET scanning) is employed to evaluate the brain uptake of intravenously injected ^{18}F-2-deoxy-D-glucose. Computerized techniques are used to calculate regional cerebral glucose metabolism ($rCMR_{glu}$) from brain uptake and time courses of plasma radioactivity and plasma glucose concentration.

Major Findings: Thirty normal volunteers without apparent primary or secondary brain disease were identified for our study. Of these, 10 were subjected to Positron Emission Tomography. The PET data, in the form of ECAT units, were converted to $rCMR_{glu}$. For some individuals over 80 years, $rCMR_{glu}$ was comparable to values in younger individuals. The data are being gathered and collated for computerized statistical analysis in relation to physiological, biochemical and psychological parameters on these subjects.

The project now is in the data gathering and collating stage. One interesting finding was made in a 59 year old male subject, who was found to be normal clinically and psychologically in all respects except for a right carotid bruit. Ophthalmodynamometry and ophthalmoplethysmography were normal and the subject was not hypertensive. However, he had a 20% reduction in $rCMR_{glu}$ in the right parietal lobe. The individual was sent to the University of Pennsylvania (W. Obrist) for the measurement of regional cerebral blood flow, which also was found to be reduced over the right parietal lobe. (Flow was measured by inhalation of ^{133}Xe.) The findings demonstrate that cerebral functional activity can be reduced in a large brain region in the absence of apparent symptoms or psychological defects. The observation serves as a cautionary note to other PET scan users, who, for example, in examining cognitive or psychiatric defects in disease states (e.g. schizophrenia), might interpret an isolated reduction in $rCMR_{glu}$ as demonstrating that the brain regions related to that defect or disease.

In addition to the above studies, the clinical group has developed expertise in validating data obtained by PET scanning, and in designing critical tests related to age-dependent norms. Dr. Robertson-Tchabo has applied the Luria psychological battery to measure regional brain defects for the first time in elderly individuals. This will establish its norm for these individuals. Dr. Duara has devised a neurological battery for the elderly so as to evaluate more quantitatively such neurological functions as coordination, motor power, reaction time and sensory acuity. Dr. Margolin has developed computerized procedures for analyzing the large quantity of data by statistical analysis. He also has established a means of stereotaxically identifing brain regions with given values of $rCMR_{glu}$, by computer assisted tomography (CAT scans) of a given brain slice with the metabolic values for the identical slice.

In addition to the ongoing protocol entitled, "Regional Cerebral Glucose Consumption in Man During Normal Aging," a protocol was written by Dr. Duara to commence next year, entitled, "Regional Cerebral Glucose Utilization in Organic Dementia of the Alzheimer Type." The purpose of this study will be to relate the cognitive, sensory and motor deficits that occur during the course of the organic dementia to the history of regional changes in glucose utilization. The dementia-related changes will be compared to observations in age- and sex-matched normal controls. To do the dementia study, we will, of course, need inpatient facilties.

Significance to Biomedical Research and the Program at the Institute: It is critical to relate cognitive function to regional cerebral metabolism in normal man as a function of age. Not only will these measures help us to understand age-related changes in the way man thinks, but they will establish reference base lines for the study of central nervous system disease. Organic dementia of the Alzheimer type afflicts as many as 15% of individuals above 65 years of age, and as such represents a major medical and social affliction. Any critical information that we can derive may help us to understand the pathogenesis and design therapies for possible treatment.

Proposed Course: Cerebral glucose utilization will be measured in screened normal subjects with positron emission tomography, as a function of age, and in subjects with organic dementia of the Alzheimer's type. Initial data will be analyzed and prepared for publication.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00127-01 LNS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Assessment of Neurochemical Markers in Relation to Age and Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edythe D. London	Sr Staff Fellow	LNS	NIA
	Steven B. Waller	Staff Fellow	LNS	NIA
Others:	Stanley I. Rapoport	Chief	LNS	NIA
	Charles L. Goodrick	Psychologist	LBS	NIA
	Donald K. Ingram	Staff Fellow	LBS	NIA

COOPERATING UNITS (if any)
Laboratory of Behavioral Sciences

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.2	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Effects of age on neurochemical markers in rodents and the correlation between these markers and behavior as well as genotype have been tested. Young adult and aged Wistar rats were tested in a 12-arm radial maze. To determine if possible age-associated behavioral alterations were correlated with defects in cholinergic, γ-aminobutyric acid (GABA)-ergic and adrenergic neurons in the cerebral cortex and hippocampus, activities of choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TH), presynaptic markers for cholinergic, GABAergic and adrenergic neurons, respectively, were assayed in these brain regions after animals were tested in the maze. Aged rats showed severe behavioral decrements as compared with young rats whereas the only significant neurochemical age effect was an increase in hippocampal TH. Maze performance was positively correlated with hippocampal CAT in aged rats and cortical GAD in young as well as aged rats. Effects of genotype and age on activities of CAT, GAD and TH in the striatum, hippocampus, cerebral cortex and cerebellum were studied simultaneously in two inbred strains of mice (C57BL/6J and A/J). Regional activities of the three enzymes were altered by age, and the effect of age varied with the genetic strain.

Project Description:

Objectives: Neurochemical sequelae of aging have been described in various species. The literature presents inconsistencies suggesting that age effects are species-specific and perhaps even strain-specific. Furthermore, the functional significance of these changes has not been adequately explored. The objectives of this project are: 1) To assess neurochemical markers in relation age, 2) to test correlations between neurochemical markers and behavior, and 3) to determine if age effects on neurochemical markers vary with genotype.

Methods Employed:

1. General methods. Wistar rats, and mice of two genetically inbred strains (C57BL/6J and A/J), were subjected to psychomotor tests at various ages. Within three days of the last test, they were killed and the brains were removed and dissected. Neurochemical markers were assayed in brain regions which were frozen and stored at -70°C until the time of assay. Correlational analyses were conducted to assess relations between neurochemical markers and individual performance in psychomotor tests.

2. Specific methods.

a. Behavioral.

(1) Radial maze performance in rats. A radial maze was constructed of translucent plastic, and consisted of a center arena approximately 60 cm in diameter from which 12 arms radiated. The food reward during all training was 0.5 ml of sweetened condensed milk, which was placed in culture dishes at the end of each arm.

Rats were placed on a food deprivation regimen that reduced their body weight to 80% of baseline for the young group and 75% of baseline for the aged group. For preliminary trials, each rat was placed in the center of the maze and allowed to obtain all the milk rewards within 15 minutes. At the end of this preliminary training, the rats responded to all arms with little hesitation. Following this training all rats were tested in the 12-arm radial maze for 15 trials conducted 5 days a week over a 3-week period.

(2) Psychomotor tests in C57BL/6J and A/J mice. Grip strength was tested as were performance on a balance beam and rotorod, free exploration, forced exploration and wheel activity.

b. Neurochemical markers. The activities of the neurotransmitter synthetic enzymes choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TH) were assayed in various brain regions of rats and mice. The activities of these enzymes reflect the integrity of presynaptic cholinergic, GABAergic and adrenergic neurons, respectively. Samples were sonicated in 5-20 volumes (w/v) of 0.05 M Tris-HCl, pH 7.4, containing 0.2% (v/v) Triton X-100, and were centrifuged at 0-4°C for 10 min at 14,000 x g. In the resultant supernatants, CAT activity was measured by the method of Bull and Oderfeld-Nowak (J. Neurochem. 19:935-947, 1971) with 60 mM choline chloride and 0.1 mM [1-^{14}C]-acetylcoenzyme A;

GAD activity was measured by the method of Wilson et al., (J. Biol. Chem. 247: 3154-3169, 1972) with 2.5 mM [1-^{14}C]glutamic acid and TH activity was assayed by the method of Coyle (Biochem. Pharmacol. 21: 1935-1944, 1972), with 50 µM L-tyrosine and 0.5 mM tetrahydropteridine cofactor. Enzyme activities were reported in terms of protein, which was assayed by the method of Lowry et al. (J. Biol. Chem. 193: 265-275, 1951).

Major Findings:

1. Behavioral.

a. Radial maze performance in rats. The performance of the aged (26 months) rats in the 12-arm radial maze was impaired markedly in comparison to the younger group (8 months). Statistically significant performance ($p < 0.05$) was achieved when an animal selected at least eight different arms on any trial from the possible twelve before making a repetition.

The age difference in the number of initial correct responses (prior to re-entering an arm) was verified statistically in a two-way (age x trials) analysis of variance with repeated measures. The main effect of age was significant, as was the interaction between age and trials ($p < 0.001$), demonstrating a faster rate of learning in the younger group. The young group demonstrated improved performance across trials, as progressively fewer choices were required to obtain all of the food rewards. In contrast, the aged group continued to exhibit a large number of responses across trials.

b. Psychomotor tests in C57BL/6J and A/J mice. Data are currently being analyzed.

2. Neurochemical markers - relation to behavior and genotype.

a. Wistar rats. The age comparisons on the specific activities of CAT, GAD and TH in the hippocampus and cerebral cortex were submitted to a multivariate analysis of variance with age as the independent variable and the enzyme activities in each site as the dependent variables. The only significant group difference was in the hippocampus, where there was a 47% increase in TH specific activity with age. Hippocampal and cortical protein concentrations were not affected significantly by age.

There were no significant correlations between maze performance and cortical TH, cortical CAT or hippocampal GAD. However, there was a significant correlation between hippocampal CAT activity and maze performance during maze trials 6-15 in the aged group ($r = 0.58$, $p < 0.05$). In addition, cortical GAD was positively correlated with performance. During trials 11-15, the correlation between cortical GAD activity and mean correct responses was significant ($r = 0.42$, $p < 0.05$). This correlation was largely due to the strong relation between these parameters in the aged group ($r = 0.68$, $p < 0.008$) where during this phase of training, a few animals were showing improvement. The relation in the young group was positive but not significant ($r = 0.38$, $p = 0.31$).

b. C57BL/6J and A/J mice.

(1) Genotype. TH activity was higher in C57BL/6J mice compared to A/J mice in all brain regions of all age groups except the striatum at 4 months, hippocampus at 18 months and the cortex at 24 months, where there were no strain-related differences.

CAT activity was higher in the C57BL/6J mice in the cortex and striatum at 4 months and the cortex at 18 months.

GAD activity of A/J mice was higher than in C57BL/6J mice in the cortex and striatum at 4 months and the cortex at 18 months. In contrast, GAD activity was lower in the cerebellum and striatum of 24 month old A/J mice than in C57BL/6J mice.

(2) Age. Striatal TH of both strains was not affected by age. TH activity increased in A/J mice between 4 and 18 months in the cortex, cerebellum and hippocampus and between 4 and 24 months in the cortex and cerebellum. TH in C57BL/6J mice increased between 4 and 18 months in the cortex and between 4 and 24 months in the cerebellum, but declined in the cortex between 18 and 24 months.

GAD activity declined between 4 and 24 months in the A/J striatum and C57BL/6J cortex, and increased between 18 and 24 months in the A/J hippocampus and between 4 and 24 months and 18 and 24 months in the hippocampus and striatum of C57BL/6J mice.

CAT activity increased between 4 and 18 months and 4 and 24 months in the cortex of both strains and in the A/J cerebellum and C57BL/6J hippocampus. Between 18 and 24 months, CAT activity was elevated in the A/J cerebellum and the hippocampus of both strains. Striatal CAT increased in C57BL/6J mice between 4 and 24 months.

Significance to Biomedical Research and the Program of the Institute:

The correlations between maze performance and cortical GAD and hippocampal CAT suggest that pharmacologic manipulations which augment central GABAergic mechanisms may enhance maze performance. They also suggest that cholinergic agents may influence cognitive behavior more in aged than in young rats. Because of the cognitive deficits that occur in human aging, such correlations may help elucidate the functional significance of postmortem neurochemical findings. Furthermore they may ultimately lead to effective pharmacotherapy for senescence-associated cognitive impairments.

Because of genetic variability in levels of neurochemical markers, it seems reasonable that age effects on these markers might vary with genotype. Differences in reported age effects on neurochemistry may result partially from use of different rodent strains by various investigators or from differences in experimental techniques. The current studies indicate that some age-related neurochemical changes are strain-specific. This underscores the importance of simultaneous studies of several genetically inbred strains or hybrid strains. Furthermore, it illustrates the limitations of extrapolations from rodents to man.

Proposed Course: Future studies will be directed at defining whether age-related changes in enzyme activities reflect changes in maximal velocities or altered substrate or cofactor affinities. In addition, analyses will be performed to detect possible correlations between regional enzyme activities and individual scores of mice in 5 psychomotor tests performed prior to sacrifice.

Publications:

Ingram, D.K., London, E.D. and Goodrick, C.L.: Age and neurochemical correlates of radial maze performance in rats. Neurobiol. Aging 2(1): 41-47, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00128-01 LNS

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Drug Pharmacokinetics, Relation to Pharmacodynamics and Senescence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I.M. Kapetanovic	Senior Staff Fellow	LNS	NIA
Others:	S.I. Rapoport	Chief	LNS	NIA
	D.J. Sweeney	Chemist	LNS	NIA

COOPERATING UNITS (if any)

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LAB/BRANCH

Gerontology Research Center, Laboratory of Neurosciences

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NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.0	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is concerned with pharmacokinetics of central nervous system drugs, the relation of pharmacokinetics to pharmacodynamics and the nature of the modifying influence of senescence. Biodisposition of haloperidol was examined in 3 and 33 month-old male Fischer-344 rats. Plasma and regional brain (striatum mesolimbus, frontal cortex and cerebellum) concentrations were measured after extraction, by gas chromatographic assay with nitrogen selective detector. Plasma elimination half-life of haloperidol was longer and plasma and regional brain concentrations were significantly higher at 6 and 9 hrs after bolus intraperitoneal injection in 33 month-old rats. During continuous intraperitoneal administration, plasma and regional brain concentrations of haloperidol were not significantly different between the two age groups. Regional brain concentrations of haloperidol did not correlate with regional dopamine receptor density.

Objectives. The elderly respond differently to various drugs than do mature adults. For example, they are more susceptible to untoward sedation with benzodiazepines and to extrapyramidal symptoms with neuroleptics, centrally-acting drugs which are widely prescribed for them. Only limited information is available, however, on the causes for the altered responses in the elderly. Response to a drug is determined by a time-dependent delivery to a target site and by target tissue sensitivity. In other words, pharmacokinetic and pharmacodynamic factors jointly govern drug responses, but the relation between pharmacokinetics and pharmacodynamics is not defined for many CNS drugs in both young or old subjects. Therefore, a multifaceted study was formulated to help elucidate relations among drug dosage, the plasma concentration, the regional brain concentration, the target site concentration and the biochemical, physiological and/or behavioral responses.

Specific aims are as follows:

1. Establish sensitive and specific analytical methodology for quantitation of drugs, metabolites and neurotransmitters in various biological tissues.
2. Describe CNS pharmacokinetics for specific drugs. Haloperidol, a butyrophenone type neuroleptic, was selected as the first drug in the study. It was chosen for several reasons: a) it is thought not to have any active metabolites thus simplifying the study of pharmacokinetic-pharmacodynamic relationship, b) its CNS pharmacokinetics is incompletely defined, c) its therapeutic action is believed to be related to dopamine receptor inhibition and therefore specific target tissues are available, and d) the elderly appear more prone to extrapyramidal symptoms and tardive dyskinesia resulting from the use of this drug.

3. Describe pharmacokinetic-pharmacodynamic relationships.

4. Examine modifying effects of age on the above.

5. Identify and study other drugs with altered responses in the elderly.

6. Study the effects of perturbation of specific pharmacokinetic factors on the overall pharmacokinetics and pharmacodynamics.

Methods Employed:

1. Experimental animals. Male Fischer-344 rats (mean lifetime 29 months, maximum lifetime 35 months) were used. This strain is commonly used in aging research and is relatively free of brain pathology.
2. Drug administration. Bolus injections were administered intraperitoneally. Continuous administration was achieved with intraperitoneally-implanted osmotic minipumps.
3. Sample collection. Blood samples were collected following decapitation. Regional brain samples were obtained following standard dissection of the appropriate areas. In the case of haloperidol, dopamine receptor-containing regions; striatum, mesolimbus (olfactory tubercle and nucleus accumbens) and frontal cortex, were dissected as was dopamine receptor-poor region, cerebellum.
4. Tissue drug and neurotransmitter concentrations. Tissue samples were homogenized and extracted with suitable solvent systems. Drug and neurotransmitter concentrations were determined with a variety of sensitive and specific chromatographic systems, namely gas chromatography (nitrogen-phosphorus and electron capture

detectors), gas chromatography-mass spectrometry-computer system, and high pressure liquid chromatography (ultraviolet absorbance and electrochemical detectors or liquid scintillation of collected fractions). For example, gas chromatographic analysis with a nitrogen selective detector was employed for quantitation of haloperidol and high pressure liquid chromatography with an electrochemical detector was used for determination of dopamine and its metabolites.

5. Response monitoring. From the biochemical standpoint, neurotransmitters and their turnover can be examined in the appropriate tissue. For example, in the case of haloperidol, tissue concentrations of dopamine and its metabolites were measured. On the physiological side, EEG, blood pressure and heart rates can be monitored. Behaviorally, appropriate tests may be used such as catalepsy which is thought to be indicative of extrapyramidal effects after haloperidol.

6. Data analysis. Mathematical modeling was achieved with the aid of MLAB, an interactive system for mathematical modeling using the DEC system 10 computer facility at NIH.

Major Findings: Biodisposition of haloperidol (HAL) was examined in mature, 3 month-old and senescent, 33 month-old male Fischer-344 rats. Following an intraperitoneal bolus injection of 0.5 mg/kg haloperidol (in an acidified aqueous solution), plasma elimination half-life was 3.6 and 2.6 hr in the senescent and mature animals, respectively. At 6 and 9 hr after injection, plasma concentrations of haloperidol were 1.5 - 2-fold higher in the senescent animals ($p < 0.05$). HAL concentrations were similar at all times in the four brain regions examined for both age groups. This lack of correlation with regional dopamine receptor density probably was due to nonspecific binding. At 6 and 9 hr after injection, regional brain concentrations of HAL were significantly higher by 1.5 - 2-fold in the senescent animals ($p < 0.05$). During continuous HAL administration (intraperitoneally implanted osmotic minipumps), however, plasma and regional brain concentrations did not differ significantly between the age groups ($p > 0.05$), and HAL concentrations were similar in the four brain regions. These results show pharmacokinetic differences between senescent and mature rats following bolus but not continuous HAL administration. An abstract of this work has been published (I. M. Kapetanovic and S.I. Rapoport, "Biodisposition of Haloperidol in Mature and Senescent Rats," Pharmacologist, 23:178, 1981).

Significance to Biomedical Research and the Program at the Institute. Due to a lack of adequate understanding of the pharmacokinetic-pharmacodynamic relationship for many CNS drugs, clinical therapy has been largely limited to empiricism. Altered responses of the elderly further complicate therapeutics in this age group. These studies should elucidate CNS pharmacokinetics and the factors which determine clinical response to HAL. Furthermore, by examining the effects of age on factors which modify the drug response, drug therapy in the elderly may be made more rational.

Proposed Course: The pharmacokinetic aspect of the HAL study will be completed. The study will be extended to steady-state conditions in order to explain different findings after bolus and continuous administration of HAL. An attempt will be made to examine specific binding of HAL in vivo. These studies will use tracer amounts of radioactive HAL isotope. A description of CNS pharmacokinetics for HAL will be formulated. The initial work will be prepared for publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00129-01 LNS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Transport Systems at the Blood-Brain Barrier

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Q.R. Smith	Staff Fellow	LNS	NIA
Others:	S.I. Rapoport	Chief	LNS	NIA

COOPERATING UNITS (if any)

-

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chloride transport across the cerebral capillary endothelium was investigated. The concentration dependencies of unidirectional ^{36}Cl influx into brain and of brain Cl content were measured at the steady state after partial replacement of body Cl with nitrate. When plasma [Cl] was varied over a 64 mM range, changes in brain Cl concentrations were proportional to plasma concentrations. ^{36}Cl unidirectional influx from plasma to brain was related linearly to plasma [Cl] over the range of 50-114 mM. In contrast to brain tissue, cerebrospinal fluid (CSF) was capable of some Cl homeostasis; ΔCSF[Cl]/Δ plasma [Cl] = 0.85. The results are consistent with passive-diffusional transport of Cl across the blood-brain barrier.

PHS-6040
(Rev. 2-81)

Objectives: The Na and Cl contents of brain may be regulated in part by carrier-mediated transport processes at the brain capillaries (blood-brain barrier) as well as by the transport systems of neuronal and glial cells and by exchange with cerebrospinal fluid (CSF). The aims of this project are as follows: 1) to measure regional cerebrovascular permeabilty to Cl in the unanesthetized rat, 2) to determine whether passive diffusion, facilitated diffusion, or active transport are involved in brain tissue Cl regulation, and 3) to determine the role of CSF in Cl distribution in the central nervous system.

Methods Employed: The concentration dependencies of unidirectional ^{36}Cl influx (plasma to brain) and of brain Cl content were measured at the steady state after partial replacement of body Cl with nitrate (NO_3). Osborn-Mendel rats were anesthetized with pentobarbital. Plasma [Cl] was set at 50 ± 2, 72 ± 2, 97 ± 1 or 114 ± 1 mmol/l (mean ± S.E.M.) by peritoneal dialysis (3 cycles, 45 min/cycle) with rat artificial interstitial fluid containing 0, 50, 110, or 180 mM Cl, respectively. Then, bilateral nephrectomy was utilized to maintain constant plasma [Cl] for 6 hr, during which time brain Cl content reached a steady-state distribution. At 1/2 hr prior to death, ^{36}Cl (18 µCi/kg) and ^{3}H-inulin (90 µCi/kg) were injected intravenously, and arterial plasma activity monitored until the rat was killed. Samples from 9 brain regions, cisternal CSF, 4 peripheral tissues, blood, and plasma were collected. ^{36}Cl and ^{3}H radioactivity were determined by dual-label liquid scintillation spectrometry. ^{36}Cl unidirectional influx and cerebrovascular permeability surface-area product (PA) were calculated for brain tissue after correction for residual vascular ^{36}Cl activity; the vascular volume of brain tissue was obtained from the ^{3}H-inulin data. Chloride in tissues and fluids was determined by amperometric titration (Cotlove, E., 1963, Anal. Chem. 35: 101-105).

Major Findings: 1) Regional permeability of the blood-brain barrier to Cl. In control rats (plasma [Cl] = 97 ± 1 mM), PA to Cl ranged from a minimum of 1.7 ± 0.1 x 10^{-5} 1/sec in the parietal lobe of the cerebral cortex to a maximum of 3.5 ± 0.4 x 10^{-5} 1/sec in the hippocampus. Assuming a brain capillary surface area of 240 cm /sec (Crone C., 1963, Acta. Physiol. Scand. 58: 292-305), the cerebrovascular permeability coefficient (P) to Cl was 0.7 - 1.5 x 10^{-7} cm/sec, comparable to that of urea and mannitol (Rapoport et al., 1979, Brain Res. 172: 354-359). The cerebrovascular permeability to Cl is similar to that of a "tight" epithelium where ions pass through the tight junctions between cells. A fraction of the Cl influx into the brain may be linked to Na transport; a significant correlation ($p < 0.05$) was found between the regional cerebrovascular permeability to Cl and the regional brain distribution of ^{3}H-ouabain (a potent inhibitor of the Na-K pump) (Donaldson et al., 1972, Can. J. Biochem. 50: 888-896).

2. Linear relation between ^{36}Cl unidirectional influx (plasma to brain) and plasma [Cl]. ^{36}Cl unidirectional influx from plasma to brain was related linearly to plasma [Cl] and extrapolated to the origin over a plasma [Cl] range of 50-114 mM. Likewise, brain PA to Cl did not show any significant concentration dependence over a 64 mM [Cl] range. The lack of a concentration-dependent PA for flux from blood to brain suggests that Cl transport across the blood-brain barrier is governed by passive-diffusional forces and not by a carrier-mediated process.

3. Lack of brain Cl homeostasis. When plasma [Cl] was reduced from 97 to 50 mM by peritoneal dialysis and when sufficient time was allowed for the attainment of a new steady state, brain tissue lost about the same percentage of Cl as did plasma (∿ 50%). Brain [Cl] varied in direct proportion to plasma [Cl]. Similar findings were obtained with skeletal muscle, liver, and heart. The data indicate that the blood-brain barrier does not regulate Cl transport nor maintain homeostatic control of brain tissue Cl content. Furthermore, the constancy of the brain Cl distribution volume (Vd) and the constancy of the PA for influx, over a 50 - 114 mM range, would indicate a concentration independent PA for outflux from brain to plasma.

4. CSF Cl Homoeostatis. The slope of the line relating CSF [Cl] to plasma [Cl] was significantly less than 1.0 (slope = 0.85 ± 0.04; $p < 0.01$). Also, the line had a positive y-intercept of 45.4 ± 3.8 mmol Cl/l CSF. The Cl Vd in the CSF (CSF [Cl]/plasma [Cl]) decreased significantly from 1.62 ± 0.06 at 50 mM plasma to 1.21 ± 0.02 at 114 mM plasma. Homeostatic control of CSF [Cl] may be a result of carrier-mediated transport of Cl from plasma to CSF at the choroid plexus (Bourke *et al.*, 1970, *J. Neurochem.* 10: 17-38; Smith *et al.*, 1981, *J. Neurochem.* in press).

An abstract of the above work has been published (Smith, Q.R. and S.I. Rapoport. Chloride movement across the blood-brain barrier: Evidence for passive-diffusional transport. Abstr. Eleventh Annual Meeting Soc. Neurosciences, 1981).

Significance to Biomedical Research and the Program at the Institute: Little is known about the mechanisms at the blood-brain barrier which regulate the ionic composition of the brain. The finding that Cl movement at cerebral blood vessels is by passive diffusion, and that cerebrovascular permeability to Cl is low, indicates that brain [Cl] is governed mainly by restricted movement of the ion across the blood-brain barrier. Pathological conditions which may damage the cerebral endothelium (e.g., Alzheimer's disease, lead encephalopathy, acute hypertension) might produce an imbalance of brain Cl.

Proposed Course: We will continue to study ionic transport at the cerebral endothelium, in relation to regulation of the brain composition and of brain neuronal function. The results on blood-brain barrier Cl transport will be prepared for publication.

Publications: None

ANNUAL REPORT OF THE CLINICAL PHYSIOLOGY BRANCH
October 1, 1980 to September 30, 1981

SUMMARY

Studies in the five sections of the Clinical Physiology Branch again demonstrate that the Branch is aptly named. Research may be characterized generally as being focused on the physiology of normal aging in experimental animals and in man. In addition, in selected areas, the effect of aging processes on the characteristic diseases of old age are being investigated. The rat is the primary experimental animal and the volunteers in the Baltimore Longitudinal Study of Aging constitute the subjects for studies in men and women. It may be noted that there is a great deal of inter-Section and inter-Laboratory collaboration of CPB scientists as well as collaboration with scientists outside the Institute. Studies from the Sections will be summarized as if they are distinct for that Section, despite the collaborative nature of many of the individual projects.

HUMAN PERFORMANCE SECTION

The Baltimore Longitudinal Study of Aging (BLSA) is largely based within the HPS, although the other four Sections of the Clinical Physiology Branch, the three Sections of the Laboratory of Behavioral Sciences, and, in a more limited way, other laboratories at the GRC also participate in the Study.

The BLSA suffered a serious loss this year in the premature death of the Coordinator of the Study, Arthur Norris, in December. The resignation of the chief of computer operations and prolonged necessary leaves of absence of the supervisor of the testing program and of the secretary (administrator) of the Human Performance Section increased the difficulties. Continuity of the ongoing testing program was maintained and some new programs have been introduced under revised governance and administrative procedures. Governance is now under a five-member Steering Committee composed of the Scientific Director, the Clinical Director, and three other senior scientists at the GRC (one clinical investigator and two psychologists). Jordan Tobin agreed to act as Chief of the Human Performance Section, thus further strengthening the BLSA, but at the cost of weakening the Metabolism Section which he left. The recruitment of Larry Brant as statistician has provided innovative techniques for the analysis of longitudinal data as well as essential statistical consultation for many scientists at the GRC.

The BLSA passed its 23rd anniversary for men and its 3rd anniversary for women. The number of women enrolled in the study is growing slowly--296 have been examined. The goal of reaching 100 women for each decade of adult life and of maintaining an equal sized sample of men cannot be met under the current testing schedule with the current staffing pattern. One of the problems in the expansion of the female study has been the success of the effort to locate and to reactivate the men who, over the years, had dropped out or formally resigned from the study. Thus, 70 of these male losses are now again active participants. Efforts at determining the alive-or-dead status of the lost subjects was extremely successful; mortality status is known for 100% of the subjects.

ENDOCRINOLOGY SECTION

The Endocrinology Section continues to examine the biochemical and physiological alterations of hormone secretion and action that occur during aging.

Aging is known to be associated with decreased hormone-sensitive lipolytic responsiveness. Accordingly, the fat cells of rat and man have been under study in an attempt to explain the mechanisms involved. The components of the fat cell lipolytic response include beta-adrenergic receptors and the enzyme adenylate cyclase, both of which are membrane bound. A number of observations on these components indicate the uniqueness of the human fat cell adenylate cyclase, particularly its dependence on guanine nucleotide. These observations question aspects of current theories concerning the mechanism of action of these regulators. The relationship between beta-adrenergic receptor number and adenylate cyclase activation has been explored using a new irreversible beta-adrenergic antagonist. A close relationship between sensitivity of hormone response and receptor number has been shown.

Dietary restriction has previously been shown by us greatly to affect the hormone sensitive adenylate cyclase. We have now shown that, while beta-adrenegic receptors and total adenylate cyclase activity are not affected by short-term starvation in rats, the sensitivity of the adenylate cyclase system is markedly (3-4 fold) increased, i.e., the dose-response to hormone is shifted to the left. This finding, indicating "tighter-coupling" of the system, has not previously been demonstrated as a mechanism for altered hormone responsiveness of any system of which we are aware.

Other aspects of the age-related alterations of hormone action have also been studied. Preliminary evidence suggests that the physio-chemical characteristics of rat fat cell membranes may change during senescence, since studies with electron spin-label probes indicate altered fluidity.

Wound healing of skin is slowed in senescent rats in which estrogen levels are low. Estrogens return the rates of healing towards those of younger animals. Other evidence shows loss of estrogen receptors from rat uterus during aging; the loss is due to decreased synthesis rate. Whether the events in skin are related to such alterations of estrogen receptors or to other factors remains to be determined.

CPB SUMMARY

The notion that age-related alterations of hormone action are invariably related to receptor changes has been shown to be simplistic. In rat parotid gland cells, alpha-adrenergic receptors are unchanged in numbers and affinity while an alpha-receptor-mediated event, potassium release, is decreased during aging. Altered calcium transport may be involved. This is yet another example of age-related alterations of hormone action that occur beyond the receptor level. On the other hand, the list of receptors that are affected during aging has been extended by demonstration that dopamine receptors in brain are decreased in the rat. The possible relationship of this change to alterations of dopamine-mediated behavior is being explored.

Work on the effects of age on the hypothalamic-pituitary-gonadal axis has continued. Efforts have been diversified in that aging effects on the pituitary-thyrotropic axis and of uremia on both pituitary-gonadal and pituitary-thyroid physiology are now in progress. We have found that pituitary cells isolated from aged rats show decreased secretion of gonadotropins in response to the hypothalamic gonadotropic releasing factor (LRH). The difference between young and aged rats persists after castration (which exposes pituitaries of both groups to increased amounts of LRH). This finding suggests that pituitary gonadotropic cells develop an intrinsic defect in secretory function with age. Measurements of hypothalamic LRH content, turnover rates of hypothalamic neurotransmitters, and characterization of pituitary membrane LRH receptors are all in progress, with promising preliminary results.

Studies are underway in men of the BLSA to define aging effects on responsiveness of the pituitary to thyrotropin releasing hormone. Repeat studies of our measurements of serum levels of gonadal steroids and gonadotropic hormones are being obtained in order to gather longitudinal data on the reproductive system. Further investigations in humans have involved determinations of the rate of metabolic disposal of glycoprotein hormones during aging and in various endocrine states.

CARDIOVASCULAR SECTION

The Cardiovascular Section designs and implements studies of aging of the cardiovascular system and excitation-contraction studies in cardiac muscle. Specific details of each project will be found in the Annual Report for that project, the number of which is listed in conjunction with the summary presented here.

Studies in Man - Z01 AG 00010-08 CPB

Ventricular Performance. The overall objectives of these studies are assessment of myocardial performance during stress. Both our intramural work and collaborative effects via contract with Johns Hopkins Cardiology Division have uncovered some new major findings. Of particular interest is the observation that, during the stress of voluntary dynamic exercise, end-diastolic volume is larger than at rest in senescent men whereas end-systolic volume is smaller than at rest in younger men. This indicates that the cardiac output increase during exercise is achieved by different mechanisms in the elderly versus the younger adult and suggests that limitation of maximum cardiac function in the elderly is not due to compromised filling but rather increased impedance to ejection and/or diminished maximum contractility.

Myocardial Blood Flow. In the past, major epidemiologic studies which have sought to identify "risk factors" for coronary artery disease, have failed to employ stress as a tool to determine the presence or absence of latent coronary artery disease. In collaboration with the Johns Hopkins Cardiology Division, monitoring the response to cardiovascular stress in men of various ages with thallium perfusion scans and electrocardiography, we have found that the prevalence of coronary artery disease in men in the 6-8th decade doubles with the results of stress testing plus resting criteria as compared with the resting criteria alone. Thus, nearly all major epidemiologic studies to date have underestimated the prevalence of cardiac artery disease. The more sensitive definition of the antemortem prevalence of coronary artery disease is a unique feature of the BLSA.

Other studies of aging in humans involve 24-hour monitoring of heart rhythm and blood pressure (Z01 AG 00033-03 CPB), peripheral blood flow, and assessment of both the acute responsiveness to and the long term effects of withdrawal of cardiac glycosides in patients with congestive heart failure (Z01 AG 00029-04 CPB).

In other areas of research, we have focused on related questions in both man and animals. It is well known that the maximum cardiovascular performance during exercise declines with advancing age. In collaboration with the Human Performance Section, we have found that failure to secrete catecholamines during exercise is not a mechanism for the decline in maximum cardiovascular function because catecholamine secretion during graded voluntary exercise increased significantly as a function of age in healthy men in the Longitudinal Aging population. One interpretation of these results is that target organ responsiveness to catecholamines diminishes. This is in agreement with previous studies in isolated rat hearts and in intact senescent dogs. Most recently, in isolated rat myocardium, in collaboration with the Laboratory of Molecular Aging and the Endocrinology Section, we have reconfirmed this diminution in the response of the senescent heart to catecholamines and, in addition, have determined that the mechanism for the age deficiency is distal to the beta-receptor and activation of protein kinase, but proximal to the effector, i.e., Ca^{2+} activation of the myofilaments.

Studies in Animal Tissues

Scattered Light Intensity Fluctuations (SLIF) Detect Intracellular Calcium Oscillations in Myocardial Tissue (Z01 AG 00035-02 CPB). Using a novel light scattering technique, we have identified cellular calcium oscillations in intact cardiac muscle. The results of these light scattering studies for the first time demonstrate that Ca^{2+} oscillations are present during the diastolic period in myocardial tissue with intact membranes. That such an oscillatory Ca^{2+} pool (myoplasmic-sarcoplasmic reticulum) exists has heretofore been unrecognized in studies of cardiac muscle. Quantitation of the frequency of the oscillations permits estimation of changes in extent of Ca^{2+} content within this pool. Over a broad range of conditions, which include the response to cardiac stimulants, the change in the frequency of SLIF varies directly with the capacity of the muscle to develop force suggesting an intimate relationship between the oscillatory "Ca^{2+} pool" and excitation contraction coupling in cardiac muscle. Thus, measurements of diastolic SLIF provide a new unique method to study fundamental mechanisms which underlie cardiac contraction both in the normal case and in disease states.

Interaction of Aging and Abnormal Loading of the Heart (Z01 AG 00037-01 CPB). Most investigations into the mechanism(s) of myocardial failure have utilized young animals in experimental disease models. This is an unrealistic approach in many ways because myocardial diseases progress with time and/or age. Utilizing older animals in experimental cardiac hypertrophy models, we have discovered new insights into cardiac decompensation in these abnormal states. While younger animals adapt to chronic volume workloads without a change in myocardial function, middle-aged animals, on the other hand, do exhibit signs of myocardial dysfunction. These age-related differences in response to enhanced cardiovascular workloads provide at least one mechanism to explain, in part, the apparent progressive worsening of some cardiac failure that occurs with disease states in aging man.

Cellular Effects of Catecholamines (Z01 AG 00032-03 CPB). Changes that occur within the cardiac cell in response to beta-adrenergic stimulation result in a dual effect on the mammalian cardiac contraction: a potentiating effect, manifest as an enhancement of twitch force, and a relaxant effect, evidenced by a decrease in the time to peak force and twitch duration, and an enhanced rate of relaxation. Although the relaxant effects can occur in the absence of twitch potentiation, it is not known whether the cellular events that result in the relaxant effect are a prerequisite for twitch potentiation in mammalian cardiac muscle. One approach toward an understanding of this would be to selectively block the relaxant effect of beta-adrenergic stimulation. We and others have demonstrated a reduction in the cAMP-dependent protein kinase-mediated stimulation of the rate of calcium accumulation in sarcoplasmic reticulum isolated from the hyperthyroid heart compared to euthyroid controls. Inasmuch as increased rate of calcium accumulation by sarcoplasmic reticulum is a potential mechanism for the cAMP-mediated relaxant effect, these findings suggested to us that the hyperthyroid heart may provide a useful model in which the twitch potentiation and changes in transmembrane ionic flux in response to beta-adrenergic stimulation might be dissociated from the usually concomitant relaxant effect.

Interaction of Age and Physical Conditioning (Z01 AG 00036-01 CPB). In our initial study of the effects of exercise on the myocardium of senescent and adult rats, we have demonstrated that two of the hallmarks of the aging rat heart, i.e., increased stiffness and prolonged contraction, would be reversed by a chronic (4-6 mo) mild exercise program. During the current year, we have expanded these studies to include variation in type, intensity and duration of exercise, and age at onset of exercise.

In addition to the aforementioned studies, we have investigated the mechanical properties of aortas from adult and senescent beagles (Z01 AG 00030-03 CPB).

CLINICAL IMMUNOLOGY SECTION

The projects in the section all have as their broad goal the elucidation of control mechanisms that modulate immune responses. The mechanisms investigated are cellular interactions, soluble factors produced by T-cells or macrophages, antibody molecules, and alterations in the composition of lymphoid tissue. The work has proceeded in the mouse, and human models in vivo, and in tissue culture.

The effect of antigen-specific cloned helper T-cells was investigated in several functional immune responses. A large repertoire of cloned helper T-cells specific for soluble or particulate antigens were established and characterized. Only about one in four of the antigen specific, I-a restricted helper T-cell clones functioned as specific helper T-cells in the in vitro response to T-dependent antigens. The effect of T-cells on regulating the in vitro response to a T-independent antigen could only be shown if a serum-free medium was used. This effect was due to soluble factors the production of which was I-a restriction but whose function was unrestricted. Several hybridomas resulting from the fusion of an antigen specific T-cell clone and BW 5142 cells are currently being characterized for functional properties.

The in vivo activity of cloned helper T-cells was established by reconstitution of nu/nu mice and by the passive transfer of a delayed type hypersensitivity reaction.

Using immunodeficient nude mice and old mice, the role of macrophages and T-cells are being explored. Macrophages can be replaced in the immune response by soluble factors obtained from macrophage cultures. T-cells which are known to be a necessary factor in the response of antibody forming cells to some antigens are also a modulating influence on B cells responding to any antigen. This is true even if the T-cells are not sensitized to the antigen and this T-cell function can be duplicated using cell free T-cell factors. In some cases, the deficient antibody response of immunodeficient old mice can be reconstituted in vitro using soluble T-cell factors.

CPB SUMMARY

The control of the natural killer cell activity of cells which are not T or B-cells has been further described. Both dietary and hormonal control of these cells have been shown. These are important findings if we are to understand the changes seen in immune function throughout lifespan. Furthermore, the finding that human monocytes have a tumoricidal activity has been an important step forward which will require more work. Monocyte function has been a difficult topic for study but because of new assays developed in our laboratory and using the cell sorter, we should be able to proceed successfully.

Certain tumor cells and lymphoid cells have receptors for immunoglobulin molecules on their membrane. The functional significance of these receptors is varied. In some cases, the antibody can imbue the cell with antigen recognizing ability. Some features of allergy may be mediated through these receptors. However, recent work in our laboratory has outlined a novel function for immunoglobulin receptors. Antibody with specificity to an effector cell can modulate the interaction between that cell and a target cell, if the target has receptors for immunoglobulin molecules. Killer cells will have augmented killing ability if antibody bridges the target and killer cell. This finding may explain some of the pathogenesis of auto-immune disorders and is a point of great interest in the immunology research community. Several laboratories have confirmed our findings. Other control mechanisms have also been outlined which have illuminated our understanding of lymphocyte tumor interactions. There is pituitary hormone control of natural killer cell activity. There are also dietary effects on cell function. These two factors are largely unappreciated in studies of host immune function although they are exceedingly important, not only in immunology but in aging research. The effects of interferon and interferon inducers have also been investigated. The findings gave us the first indication that not all killer cells are lymphocytes. Because of differential effects of modulating agents, it became apparent that some killer cells are monocytes. This has led to our increasing interest in monocytes as effectors of immune function. With new techniques, we have been able to isolate monocytes and define their function in several in vitro assays. These studies have described the interaction between monocytes and B lymphocytes in an antibody formation system. The monocytes role in antibody formation is apparently not appreciated and this is an exciting new venture for the section.

The outline of human immune function is continuing. The features described this past year apply to T-cells; natural killer cells, monocytes, and granulocytes. Natural killer cells remain constant in their level of activity throughout age. There is a suggestion of a correlation between high levels of natural killer activity and survival.

Using monoclonal anti T-cell antisera and the laser cytofluorograf, we have for the first time demonstrated changes in the human T-cell population with age. Techniques prior to this time have not been sensitive or complete enough to allow a definitive study of human T-cells. Whereas the functional aspects of T-cells have been shown to decrease with age, only now can we see that this is due to a decrease in the T-cell population with a shift in subpopulation representations. Human natural killer cells do not change their function levels with age, nor do human B-cells. However, the B-cell data must be re-evaluated in terms of the monocytes controlling effects on B-cell function. Monocytes can be enriched and purified from the peripheral blood cell population by various physical separation methods. The significance of this finding on the interpretation of certain B-cell assays is great. The mitogen pokeweed has been found to bind specifically to monocytes rather than B-cells and the monocytes then influence B-cell differentiation. Granulocyte function, depending on the assay, has several age-related changes. Metabolic activity remains stable while the induction of superoxide production and the ability to kill bacteria both decline with age.

There are studies in both mice and humans on granulocyte function in host defense mechanisms. Results are similar in that the ability of phagocytic cells in old mice or elderly humans to kill a live bacteria declines with age. The reason for this may reside in the observation that granulocytes from elderly individuals cannot generate high levels of an enzyme which is crucial in the bacterial killing mechanism. A study of the effects of immunization with pneumococcal polysaccharide with subsequent challenge of immunized mice with the live organism has shown an interesting pattern. Whereas the antibody response is lower in the very young and very old mice, the old mice can withstand the challenge with live bacteria as well as young adult mice. Very young mice cannot survive a challenge even if immunized.

METABOLISM SECTION

A systematic review of the world's literature on the impact of obesity on mortality has been completed. There are over 40 studies in which population samples have been recruited and followed. Obesity was assessed in various ways, but generally by some index combining height and weight. It is clear from these diverse studies that minimal mortality occurs at a level of overweight in excess of that indicated in the commonly-used desirable weight tables of 1959. Possible artefacts or defects in study design or associations of obesity with other variables do not seem to explain these findings. New desirable weight tables are indicated. The deleterious effects of obesity, if anything, diminish with increasing age.

Obesity is unquestionably associated with increased likelihood of developing diabetes mellitus. We have studied a well-characterized population, the Pima-Papago tribe of Southwest American Indians, who show a remarkably high prevalence of obesity and diabetes. They were compared to an equally obese select group of Caucasians and to lean Caucasians. The insulin-infusion euglycemic clamp technique was used.

CPB SUMMARY

The kinetics of insulin distribution and destruction did not seem to be influenced by race or by obesity. While obesity was associated with a decreased sensitivity to insulin, again there was no significant race effect.

Of interest was definite evidence of suppression of beta cell secretion of insulin by the exogenous insulin infusion. This effect occurred at physiological levels of insulin in peripheral blood. The ambient insulin concentration in the immediate environment of the beta cell must, in fact, be many times higher than the concentration of arterial insulin perfusing the cell. The suppression of endogenous basal insulin secretion under these circumstances suggests that the micro-anatomy of the cells of the islet are even more complex than had been thought. The alpha cells were also suppressed by the insulin infusion.

A study of radiographic heart size was conducted in order to test the commonly-used clinical rule that a 50% cardio-thoracic ratio was the upper limit of normality for all ages. Cross-sectional age differences in cardiac and thoracic diameters as well as longitudinal age changes (over an average period of 11 years) in those measurements were assessed. It was found that in the absence of clinical evidence of heart disease, only one subject had a CTR greater than 50%--a 95-year-old man. Thus, it is reasonable to stay with the 50% rule, i.e., no age adjustment of normative criteria is justified.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00001-11 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Metabolic Studies of Aging and Obesity in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dariush Elahi	Staff Fellow	CPB	NIA
Other:	Jordan Tobin	Chief, HPS	CPB	NIA
	Reubin Andres	Chief	CPB	NIA

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LAB/BRANCH
Clinical Physiology Branch

SECTION
Metabolism Section

INSTITUTE AND LOCATION
NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	0.8	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Pima Indian population is characterized by a strikingly high prevalence of obesity and by the highest recorded prevalence of diabetes in the world. A group of non-diabetic obese Indians were compared to a group of Caucasians who were matched for age, degree of obesity, sex, and glucose tolerance status. The obese Caucasians were then further matched by a group of lean Caucasians of similar age, sex, and glucose tolerance status. Obese subjects of both races had reduced insulin sensitivity as assessed by the euglycemic clamp technique. There was no significant racial difference. The kinetics of insulin distribution and destruction was not influenced in a major way by race or obesity. Both alpha cells and beta cells of the islets of Langerhans were inhibited by the induced hyperinsulinemia. The plasma glucagon levels remained suppressed long after the plasma insulin levels had returned to basal. Plasma C-peptide reactivity however showed a more rapid recovery from insulin suppression.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Since the Indian population is characterized by very high prevalence of both obesity and diabetes, it was intended that lean and obese Indians would be compared to lean and obese Caucasians with respect to insulin sensitivity. Nondiabetic individuals of both races would be studied. Would the Indian subjects show greater insensitivity to insulin than Caucasian subjects of similar age, obesity, sex distribution, and glucose tolerance status? A further objective in these studies was to examine the controversial issue of negative feedback control (or "short-loop" feedback) of insulin on its parent cell, the beta cell. Evidence in the literature on rodents and on man is not persuasive on this point--at least with insulin levels within the physiologic range.

Methods Employed: The euglycemic insulin infusion glucose clamp technique was used to create a steady state of hyperinsulinemia with maintained euglycemia. Plasma insulin, glucagon, and C-peptide were analyzed by radioimmunoassay. Subjects were residents of the Gila River Indian Community (Pima Tribe), volunteers in a behavioral study of obesity at the Johns Hopkins University, and volunteers in the Baltimore Longitudinal Study of Aging.

Major Findings: Euglycemic clamp studies have been performed on 24 obese Indians and 41 lean and obese Caucasians. It was not possible to obtain lean volunteers of the Pima Tribe. Comparisons then can be made between obese Pima and Caucasians and between lean and obese Caucasians. The plasma insulin responses to the initiation and maintenance of the insulin infusion and to its discontinuation were very similar in the three groups of subjects. Thus, there were no major differences in the kinetics of insulin distribution or destruction attributable to race or to obesity. Sensitivity to insulin is, as expected, lower in obese than in lean subjects, but no racial differences were present in these groups of matched subjects.

An interesting finding in these studies is that there is a very definite inhibition of endogenous insulin secretion (judged by plasma C-peptide reactivity) in all subject groups. The finding is of significance in that the hyperinsulinemia induced in these studies is well within the physiologic range (100 μU/ml) and the inhibition of insulin secretion is quite large (50% reduction in basal insulin secretion rate). Thus, among the complex control factors for insulin secretion must now be added short-loop negative feedback control. Recovery from the inhibition began to occur shortly after the insulin infusion was discontinued.

Similar to this beta cell inhibition was the fall in plasma glucagon levels during the clamp procedure. Thus, alpha cell suppression also occurs. This suppression is longer-lasting than that of the beta cell.

The implications of these effects are of interest in terms of the micro-anatomy of islet structure. The ambient insulin concentration in islet interstitial fluid even under basal conditions must be many-fold higher than the inflowing arterial insulin levels under conditions of the clamp. This suggests that there might be a beta cell membrane orientation with insulin-secreting areas distinct from areas containing insulin receptions.

Significance to Biomedical Research and the Program of the Institute: Aging in man is associated with decreasing glucose tolerance and with increasing obesity. The link between obesity and diabetes is well-known but the mechanisms relating these variables to each other and to aging are unclear. The present studies add strong evidence in favor of an additional physiological control factor to the already complex homeostatic mechanisms for glucose control--a factor which has been controversial. A better understanding of insulin physiology should lead eventually to techniques to minimize possible deleterious effects of a diminishing ability with advancing age in controlling the blood glucose level.

Proposed Course: A manuscript describing these studies has been submitted for publication. Further studies on the Pima Tribe should include the use of the hyperglycemic glucose clamp technique with concomitant administration of tritiated glucose tracer to evaluate islet cell function and hepatic responses to hyperglycemia and hyperinsulinemia.

Publications:

Rowe, J. W., Shelton, R. L., Helderman, J. H., Vestal, R. E., and Robertson, G. L.: Influence of the emetic reflex on vasopressin release in man. Kidney Int. 16: 729-735, 1979.

Rowe, J. W., Tobin, J. D., Rosa, R. M., and Andres, R.: The effect of experimental potassium deficiency on glucose and insulin metabolism. Metab. 29: 498-520, 1980.

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Tobin, J. D.: Physiological indices of aging. In Marois, M. (Ed.): Aging: a challenge to science and society, Vol I Biology. London, Oxford University Press, 1981, pp. 286-295.

Andres, R.: Problems in the study of human aging. In Schimke, R. T. (Ed.): Biological Mechanisms in Aging. National Institutes of Health Publication No. 81-2194, 1981, pp. 696-700.

Hershcopf, R. J., Elahi, D., Raizes, G. S., Schocken, D. D., Tobin, J. D., and Andres, R.: Longitudinal changes in serum cholesterol in man: an epidemiologic search for an etiology. J. Chronic Dis. (In Press)

Costa, P. T., Jr., McCrae, R. R., Andres, R., and Tobin, J. D. Hypertension, somatic complaints and personality. In Elias, M. F. and Streeten, D.H.P. (Eds.): Hypertension and Cognitive Processes. Mt. Desert, Beech Hill Publishing Co., 1980, pp. 95-110.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00002-19 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Gastrointestinal Mediation of Insulin Release

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dariush Elahi	Staff Fellow	CPB	NIA
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Clinical Physiology Branch

SECTION
Metabolism Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	0.5	2.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of gastric inhibitory polypeptide (GIP) in glucose homeostasis has been further investigated in dog and man. The studies in the dog showed that the metabolic clearance rate of GIP was increased by hyperglycemia whether or not hyperinsulinemia was present, but that hyperinsulinemia alone did not influence the clearance rate. The suggestion that GIP might have an inhibitory effect on hepatic glucose output was not confirmed in these studies.

Studies in man have been directed at the possible effects of hyperglycemia and hyperinsulinemia on the secretion of GIP in response to a fat meal. Ingestion of fat leads to elevation of the plasma GIP level and this elevation is uninfluenced by subsequent increase either in plasma glucose or in plasma insulin levels. However, the establishment of hyperglycemia and hyperinsulinemia prior to fat ingestion markedly decreases the GIP response to fat ingestion; hyperinsulinemia alone has no effect. The fat-stimulated GIP has no effect on plasma glucagon levels.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: (1) To study the effects of ambient plasma glucose and insulin levels on (a) metabolic clearance of GIP from plasma and (b) fat-stimulated GIP release. (2) To study the direct effect of GIP on hepatic glucose production.

Methods employed: (1) Dog Studies. Metabolic clearance rate of GIP is measured by following plasma levels after GIP infusion. Hepatic glucose produced is calculated using the tritiated glucose infusion technique. Hyperglycemia is induced by intravenous glucose infusion; endogenous hyperinsulinemia is allowed to occur in one series of studies but is prevented in another series by infusion of an insulin-specific somatostatin analog.

(2) Human Studies. Endogenous GIP secretion is assessed following ingestion of a triglyceride emulsion. The effects of hyperglycemia and of hyperinsulinemia are examined with the hyperglycemic and euglycemic (insulin-infusion) glucose clamp methods.

Major Findings: (1) Dog Studies. Metabolic clearance rate of GIP can be calculated following single-shot infusion of GIP. The t 1/2 of GIP under basal conditions was 34 minutes. Hyperinsulinemia without hyperglycemia does not influence GIP clearance significantly (t 1/2 = 34 min). However, hyperglycemia with consequent hyperinsulinemia or hyperglycemia alone (during somatostatin blockade of insulin secretion) increased GIP clearance significantly (t 1/2 = 20 and 23 minutes, respectively). GIP infusion did not influence the rate of endogenous glucose production.

(2) In man, fat-stimulated increase in plasma GIP levels were not influenced by subsequent increases in plasma glucose or insulin levels. However, if hyperglycemia was established with the glucose clamp techniqe (and consequent endogenous hyperinsulinemia) prior to fat ingestion, then the plasma GIP response to the fat was markedly blunted. Hyperinsulinemia without hyperglycemia does not influence the GIP response to fat ingestion. The fat-stimulated GIP is not glucagonotropic.

Significance to Biomedical Research and the Program of the Institute. Aging is characterized by a loss of homeostatic control of physiological processes. Among these is the plasma glucose level, which must be maintained within a narrow range in order to avoid the dangers of hypoglycemia and of hyperglycemia. The regulation of the plasma glucose level is under complex neuro-endocrine control mechanisms. Among these is the gastro-intestinal hormone, gastric inhibitory polypeptide (GIP). Studies in the Metabolism Section have been directed at elucidating some of the physiological complexities underlying GIP secretion and that hormone's effect as an insulin secretogogue.

Proposed Course: By combining the glucose clamp technique with that of tritiated glucose infusion in man, the effect of hyperglycemia and of insulin on glucose production and on total body glucose utilization will be studied. In addition, an ongoing series of studies of the effect of four different levels

Proposed Course (continued)
of hyperglycemia on insulin secretion will be completed on participants in the Baltimore Longitudinal Study of Aging. These studies will cover the entire adult age span.

Publications:

Elahi, D., Andersen, D.K., Brown, J.C., DeBas, H.T., Hershcopf, R.J., Raizes, G.S., Tobin, J.D., and Andres, R.: Pancreatic alpha- and beta-cell responses to GIP infusion in normal man. Am. J. Physiol. 237: E185-E191, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00019-01 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effect of Dietary Chromium on Glucose Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jane Potter	Medical Officer	CPB	NIA
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Other:	Dariush Elahi	Staff Fellow	CPB	NIA
	Jordan Tobin	Chief, HPS	CPB	NIA
	Reubin Andres	Chief	CPB	NIA

COOPERATING UNITS (if any)

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Walter Mertz, Human Nutrition Ctr., Nutrition Institute, Beltsville, MD

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Clinical Physiology Branch

SECTION
Metabolism Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.7	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is designed to explore the effect of dietary chromium supplementation on the metabolism of glucose in individuals with impaired glucose tolerance utilizing the hyperglycemic glucose clamp technique. The erythroid insulin radioreceptor is used to explore the possibility that chromium acts at the level of the receptor to promote glucose metabolism. Chromium levels in serum and urine in the fasting and hyperglycemic state are measured using electron absorption spectroscopy.

In a separate phase of the study, associations among the variables age, sex, coronary heart disease, and fasting chromium levels are being examined.

Project Description:

Objectives: Studies in experimental animals and man suggest that chromium is essential to normal glucose homeostasis. The present study is designed to determine the effect of dietary supplementation with this mineral on tissue sensitivity to insulin as assessed by the hyperglycemic glucose clamp technique. Although chromium is thought to act through an interaction with the insulin receptor, studies to date have not explored this possiblity during chromium supplementation. There is presently no normative age data on serum and urine chromium levels. Techniques for accurate measurement of serum and urine levels of chromium have recently been developed, and will be used in this study to establish age and sex adjusted normal serum levels. Chromium has also been postulated to influence the development of coronary heart disease through its effect on lipid metabolism. This study will examine fasting chromium levels in individuals with and without coronary heart disease and serum lipid levels, prior to and following chromium supplementation.

Methods employed: Individuals with impaired glucose tolerance (National Diabetes Data Group criteria) are being supplemented in their normal diet by taking 200 μg of chromium chloride daily for three months. Two methods are being used to assess the effect of this chromium supplementation on tissue sensitivity to insulin. The hyperglycemic clamp technique is used to determine an overall index of glucose metabolized (M), an indicator of tissue sensitivity to insulin. Paired hyperglycemic clamp studies are performed prior to and after chromium addition. The erythrocyte radioreceptor assay is employed as another measure to evaluate changes in sensitivity to insulin in response to chromium supplementation. Recent studies have demonstrated that these insulin receptors share most of the characteristics of insulin receptors in other tissues. Possible alterations of insulin receptor number and affinity will be evaluated. The receptor assay involves separation of erythrocytes by a Ficoll-hypaque gradient and incubation with ^{125}I porcine insulin. Scatchard analysis of receptor data will be performed using computerized curve fitting techniques. Serum and urine chromium content is assayed before and after chromium supplementation. The chromium content of these samples is measured using a continuum source, wavelength modulated atomic absorption spectrometer. Lipid profiles obtained during the study include total cholesterol, LDL, HDL, and total triglyceride.

Major Findings: Subjects with impaired glucose tolerance have been selected, have undergone initial hyperglycemic glucose clamp study and are presently receiving a supplement of inorganic chromium. After the period of supplementation, individuals will return for the second of their paired studies. Approximately half of the fasting samples have been collected.

Significance to Biomedical Research and the Program of the Institute: There is a remarkably high prevalence of abnormal glucose tolerance in the older population of the United States. This study explores a possible role for nutritional deficiency of chromium (or, alternatively, a pharmacological role for chromium administration) in the impaired glucose tolerance of old age. In addition, the studies will establish normative serum chromium levels.

Proposed Course: The studies with the clamp technique and the erythrocyte receptor are currently in progress and should be completed in the next year. Collection of samples of urine and plasma for the normative data will be completed about the same time.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00020-01 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effect of Age and Heart Disease on the Cardiothoracic Ratio

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jane Potter	Medical Officer	CPB	NIA
Other:	Dariush Elahi	Staff Fellow	CPB	NIA
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COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Physiology Branch

SECTION
Metabolism Section

INSTITUTE AND LOCATION
NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study examines the reliability of the cardiothoracic ratio in the diagnosis of heart disease in old men. Members of the Baltimore Longitudinal Study of Aging had annual or semiannual chest x-rays as part of their health screening tests between 1958 and 1975. Measurements of cardiac diameter and of thoracic diameter were made on 1124 P-A chest x-rays from 243 individuals. Subjects ranged in age from 21 to 95 years and were categorized according to the presence or absence of heart disease. Cross-sectional age decade means for the measured variables and for the cardiothoracic ratio were calculated. Longitudinal changes were calculated. Future plans include correlating the observed cross-sectional differences and longitudinal changes with anthropometric measurements, body mass index and blood pressure. A mortality analysis will also be performed.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Cross-sectional studies of older people have suggested that using a cardiothoracic ratio of 50% overdiagnoses cardiomegaly in 10% of healthy men and in 20% of healthy women over age 60. This study is designed to test these findings using both cross-sectional and longitudinal chest x-ray measurements and a battery of clinical tests useful in the diagnosis of heart disease. Observed changes in thoracic diameter will be correlated with changes in certain anthropometric measurements. Change in heart size and cardiothoracic ratio will be correlated with changes in blood pressure and with body mass index. Finally, the 49 individuals from this study who had died by the time of this analysis will be compared to survivors to determine if measured variables have an effect on mortality.

Methods employed: Chest x-rays are standard six-foot postero-anterior studies. Measurements from the x-ray films were made using a standard radiologic technique. Body measurements and blood pressures were obtained on the same vists as x-rays. Subjects are male volunteers of the Baltimore Longitudinal Study of Aging.

Major Findings: Subjects were grouped into four age groups: 21-51, 60-69, 70-79, and 80-95 yr. For cross-sectional analysis, the x-ray obtained at the last visit was used. For longitudinal analysis, slopes were computed for each subject for each variable and subject's mean age was used to place him in one of the four age categories. Analyses were separately carried out for those with and without clinically identifiable heart disease. Subjects with heart disease were limited to the oldest three age groups.

1. Cross-sectional analyses (mean ages 43, 65, 75, and 85 yr). (a) Cardiac diameter was greatest in the 75-yr age group and least in the 43-yr group with the 65 and 85 yr groups intermediate. (b) Thoracic diameter was essentially the same in the 43 and 65 yr groups, declined somewhat in the 75-yr group, and was significantly less in the oldest group. (c) Thus, the cardiothoracic ratio was progressively and significantly greater with age--due initially mainly to increased cardiac diameter but due to decreased thoracic diameter in the 85-yr group. Subjects with heart disease had greater CTR as expected in each age group.

Of importance is the fact that only one subject in the entire group of normal (i.e., nonheart disease) subjects had a CTR greater than 50%, a 95-yr old man. Thus, at least into extreme old age, the 50% CTR limit of normality--a value derived from a study of young and middle-aged adults--should not be modified for age in men.

2. Longitudinal studies. The subjects were studied on the average over a period of 11 years. There are well-recognized problems in the interpretation of cross-sectional data, especially in old subjects. Cohort differences and selective mortality may lead to misinterpretation of age differences as being synonymous with age changes. It is therefore encouraging (methodologically speaking) that, at least for the variables measured in this study, the longitudinal changes closely reflect the cross-sectional differences. In general, even in subjects without heart disease, cardiac diameters rose significantly in all age groups. Thoracic diameters increased in the two younger age groups, was unchanged in the 70's and decreased in the 80's. As a result of these changes in CD and TD, CTR ratios increase throughout middle and old age.

Significance to Biomedical Research and the Program of the Institute: Many clinical standards for normality and abnormality have not been specifically tested for application to the older population. This study tests the specificity of one such commonly used clinical parameter. Autopsy and echocardiographic studies have suggested that there is an increase in left ventricular mass in normal old age, except perhaps in the very oldest age group. This study supports those findings using a very different technique of measurement. It is clear that for men, the commonly used upper limit of normality for the cardiothoracic ratio of 50% should not be modified for age.

Proposed Course: Correlations of the x-ray variables with blood pressure and with certain anthropometric variables will be carried out. The possible predictive value of the CTR for mortality will be assessed. In addition, (although a number of years will have to pass to accomplish this), similar analyses for our female participants will be carried out.

This work was presented at the Congress of the International Association of Gerontology in Hamburg, Germany, July, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00201-01 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Obesity on Mortality

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reubin Andres	Chief	CPB	NIA
Other:	Dariush Elahi	Staff Fellow	CPB	NIA
	Jordan Tobin	Chief, HPS	CPB	NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Physiology Branch

SECTION
Metabolism Section

INSTITUTE AND LOCATION
NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obesity is generally considered to be one of the major health hazards in developed societies. Since obesity is one of the controllable risk factors, body weight goals recommended to health professionals and to the general public (in the form of desirable body weight tables) are of great significance. Weight goals in most common use are derived from actuarial studies of mortality conducted from the 1930's to the 1950's. Since that time, however, a large number of additional studies have been reported. There is strong agreement among these world-wide studies that minimal mortality occurs over a rather broad range of body weight. This range of low mortality includes the upper half of the range in the widely-used 1959 Tables and even extends somewhat beyond those limits. The lower half of the "desirable" weight range is, in fact, undesirable in many of the studies; mortality is frequently excessive in those leaner subjects. Since even moderate obesity clearly is associated with an increased incidence of a number of life-shortening illnesses and of other risk factors (diabetes, hypertension, hyperlipidemia, and uterine carcinoma, for example), the lack of effect on total mortality must indicate that there are counterbalancing beneficial associations which are not understood and which deserve further study.

Project Description:

Objectives: Obesity has been called the major health hazard in the U.S. Since it is a variable which is controllable by the individual, it behooves the medical profession to define (with clarity) reasonable weight goals for the public. Present goals were set by the creation of tables of Desirable Weight based upon the results of an actuarial study reported in 1959. There are now a large number of diverse studies carried out mainly in the U.S. and Europe which offer evidence on one of the "end-points" of the obesity hazard, namely, mortality. The goal of this research is to conduct, as a first step, a critical review of the world's literature on the effect of obesity on mortality in order to aid in the construction of new weight goals.

Methods Employed: (1) Literature review supplemented by personal communications with scientists who have conducted but not yet fully reported studies on obesity and mortality. (2) Assessment of obesity levels in the Baltimore Longitudinal Study of Aging by the usual height-weight measurements and by more complex anthropometric techniques.

Major Findings: It is difficult to define an "acceptable" study in this area. There are problems in the initial recruitment and representativeness of the population at risk, in the accurate estimation of obesity, in complete follow-up of the subjects, in the measurement, analysis, and reporting of associated risk factors, and in determination of specific causes of death. Nevertheless, one must go with the best evidence available. There are now some 40 studies which offer data on the primary relationship of obesity and mortality. They cover most countries in Europe, the U.S., Australia, and Japan. Most were published since 1970. Follow-up periods were sometimes brief but generally about 10 years. Obesity assessment was generally based upon height and weight only, but a few studies also measured skin-fold thickness. Men aged 40-59 years are most commonly the population studied, but there are also a number of studies of special aged groups, and a lesser number of female populations. The number of subjects studied range from several dozen to several million, but is generally in the several thousand range. Subjects include samples of total communities, a variety of industrial and civil service employee groups, institutionalized elderly, university faculty, self-recruited volunteers, etc.

The diversity of studies adds strength to the major conclusion reached, since the results are so strongly in agreement. That conclusion is that minimal mortality occurs at a level of obesity distinctly in excess of the range of weights in the 1959 Desirable Weight tables. It is rare to find an increase in mortality at a weight below about 20-30% in excess of the middle of the weight range in the 1959 tables. Furthermore, weights below the mid-point of the 1959 tables (but still "desirable" according to those tables) are almost invariably associated with a higher mortality than weights which, up to now, have been considered to be in the mildly or moderately obese range.

Analysis of the results in the Baltimore Longitudinal Study on Aging thus far have been limited to obesity measurements based on height and weight only. They confirm the results noted above. Derivation of a formula to compute percent body fat based upon simple anthropometric measurements has been accomplished and this more accurate index of obesity will be used in future analyses.

Significance to Biomedical Research and the Program of the Institute: The American population is characterized by an increasing adiposity from the early adult to the middle-aged years in both men and women. Since body fat can be modified by changes in total caloric intake and expenditure, it is important to set weight goals for the public which are based upon the best available scientific evidence. A reconsideration of weight standards is called for now because of the large number and diversity of reports now available.

Proposed Course: The literature on the effects of obesity on specific causes of death will be critically reviewed. Possible confounding variables will be examined. For example, cigarette smokers weigh less than nonsmokers; thus, the apparent advantage of mild obesity over mild leanness may be attributable to the different percentages of smokers in the two groups.

Publications:

Andres, R.: Effect of obesity on total mortality. Int. J. Obesity 4: 381-386, 1980.

Andres, R.: Influence of obesity on longevity in the aged. In Marois, M. (Ed): Aging: a challenge to science and society, Vol. I Biology. London, Oxford University Press, 1981, pp. 196-203.

ENDOCRINOLOGY SECTION

Annual Report Summary for October 1, 1980 to September 30, 1981

The Endocrinology Section continues to examine the biochemical and physiological alterations of hormone secretion and action that occur during aging.

Aging is known to be associated with decreased hormone-sensitive lipolytic responsiveness. Accordingly, the fat cells of rat and man have been under study in an attempt to explain the mechanisms involved. The components of the fat cell lipolytic response include β-adrenergic receptors and the enzyme adenylate cyclase, both of which are membrane bound. A number of observations on these components indicate the uniqueness of the human fat cell adenylate cyclase, particularly its dependence on guanine nucleotide. These observations question aspects of current theories concerning the mechanism of action of these regulators. The relationship between β-adrenergic receptor number and adenylate cyclase activation has been explored using a new irreversible β-adrenergic antagonist. A close relationship between sensitivity of hormone response and receptor number has been shown.

Dietary restriction has previously been shown by us greatly to affect the hormone sensitive adenylate cyclase. We have now shown that, while β-adrenergic receptors and total adenylate cyclase activity are not affected by short-term starvation in rats, the sensitivity of the adenylate cyclase system is markedly (3-4 fold) increased, i.e., the dose-response to hormone is shifted to the left. This finding, indicating "tighter coupling" of the system, has not previously been demonstrated as a mechanism for altered hormone responsiveness of any system of which we are aware.

Other aspects of the age-related alterations of hormone action have also been studied. Preliminary evidence suggests that the physio-chemical charactertistics of rat fat cell membranes may change during senescence, since studies with electron spin-label probes indicate altered fluidity.

Wound healing of skin is slowed in senescent rats in which estrogen levels are low. Estrogens return the rates of healing towards those of younger animals. Other evidence shows loss of estrogen receptors from rat uterus during aging; the loss is due to decreased synthesis rate. Whether the events in skin are related to such alterations of estrogen receptors or to other factors remains to be determined.

The notion that age-related alterations of hormone action are invariably related to receptor changes has been shown to be simplistic. In rat parotid gland cells, α-adrenergic receptors are unchanged in numbers and affinity while an α-receptor-mediated event, potassium release, is decreased during aging. Altered calcium transport may be involved. This is yet another example of age-related alterations of hormone action that occur beyong the receptor level. On the other hand, the list of receptors that are affected during aging has been extended by demonstration that dopamine receptors in brain are decreased in the rat. The possible relationship of this change to alterations of dopamine-mediated behavior is being explored.

Work on the effects of age on the hypothalamic-pituitary-gonadal axis has continued. Efforts have been diversified in that aging effects on the pituitary-thyrotropic axis and of uremia on both pituitary-gonadal and pituitary-thyroid physiology are now in progress. We have found that pituitary cells isolated from aged rats show decreased secretion of gonadotropins in response to the hypothalamic gonadotropic releasing factor (LRH). The difference between young and aged rats persists after castration (which exposes pituitaries of both groups to increased amounts of LRH). This finding suggests that pituitary gonadotropic cells develop an intrinsic defect in secretory function with age. Measurements of hypothalamic LRH content, turnover rates of hypothalamic neurotransmitters, and characterization of pituitary membrane LRH receptors are all in progress, with promising preliminary results.

Studies are underway in men of the Baltimore Longitudinal Study to define aging effects on responsiveness of the pituitary to thyrotropin releasing hormone. Repeat studies of our measurements of serum levels of gonadal steroids and gonadotropic hormones are being obtained in order to gather longitudinal data on the reproductive system. Further investigations in humans have involved determinations of the rate of metabolic disposal of glycoprotein hormones during aging and in various endocrine states.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00011-09 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors, and aging I. Aging and hormone-sensitive adenylate cyclase.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	C. R. Schneyer	Clinical Associate (EOD 7/1/80)	CPB	NIA
	R. I. Gregerman	Chief, Endocrinology Section	CPB	NIA
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COOPERATING UNITS (if any)

Department of Surgery, Baltimore City Hospitals

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies deal primarily with influences of age on the biochemistry of hormone-sensitive adenylate cyclase in a variety of tissues. The purpose of these studies is to explore the mechanisms of age-related alterations of hormone responsiveness and biological membranes, with special emphasis on the relationship between adenylate cyclase and hormone receptors. Other aspects include ongoing literature review of the field of aging and hormones in general and of certain aspects of clinical medicine as they relate to aging and endocrinology.

Project Description:

Objectives: The experimental portion of this project explores age-related effects on the hormone-sensitive adenylate cyclase system. The action of many hormones is at the cell surface where each hormone combines with its specific receptor protein and thereby initiates a series of reactions. The hormones which act in this manner include peptides and proteins (glucagon, vasopressin, vasoactive intestinal polypeptide, thyrotropin, luteinizing hormone, etc.) and small amines such as the catecholamines (epinephrine, norepinephrine, dopamine, serotonin, histamine). The result of the hormone-receptor interaction is activation of the membrane-bound enzyme, adenylate cyclase (AC) which catalyzes the conversion of adenosine triphosphate (ATP) to the cyclic nucleotide, adenosine-3'-5'-monophosphate (cAMP; cyclic AMP). This key compound in turn phosphorylates and thereby activates a number of enzymes (protein kinases), an event which initiates or accelerates many cellular processes (e.g., lipolysis, the breakdown of fat; the permeability of membranes to water; relaxation of smooth muscle in bronchi, etc.). The intracellular concentration of cAMP is regulated by phosphodiesterases, enzymes which degrade cAMP. Recently, it has become apparent that some hormones can initiate inhibition action on the AC system.

The biochemistry of adenylate cyclase (AC complex) has been extensively studied in recent years. The characteristics of the receptor proteins have been partially elucidated and methods devised for quantitation of receptors. A key protein which interacts with both the receptor and the catalytic unit of the cyclase is called the GTP-binding regulatory protein (also termed G/F or N protein). This regulator has now been purified to homogeneity and shown to be a vital component of the AC system in many--if not all--cells. The AC complex is imbedded in and its activity determined by the lipid matrix of the cell membrane.

A number of age-related alterations of hormone action are known in which changes of the AC system may play a role. It has been an objective of our laboratory over the past few years to a) identify the AC alterations that are age related, b) elucidate the biochemical mechanisms by which these age-related changes occur. In this regard we have developed methods for quantitation of receptors and of adenylate cyclase. The role of activator proteins has been extensively studied. Since hormone receptors and AC are part of the cell membrane, our work is a probe of age effects on cell membranes.

Methods employed: Tissues used are from experimental animals (mainly the rat) and from man (surgical specimens). Tissue homogenates, isolated cells, and cell membranes are employed, cells being isolated by enzymatic (collagenase) digestion and membranes by density gradient centrifugation, etc. Adenylate cyclase is quantitated by a labeled substrate assay in which α-^{32}P ATP is converted to a α^{32}-cAMP and the latter quantitated after isolation on columns. Use of two marker isotopes (^{3}H-cAMP and ^{14}C-cAMP) enables precise correction of losses due to destruction during incubation and chromatography. Receptor quantitation utilizes labeled hormone antagonists. Protein factors are isolated by standard techniques of protein fractionation (gel and ion-exchange chromatography, etc.).

Major Findings: Biochemistry of the Adenylate Cyclase System

1) Human fat cell cyclase. We have previously studied the human AC system and described a number of differences from that of the rat (2, 6). As part of the further characterization of this system in anticipation of age-related measurements, we have further examined the quanine nucleotide requirement of the catecholamine (hormone) stimulated enzyme.

In previous work we showed that GTP was essential for hormone stimulation of the human AC system. Purified membranes showed an absolute requirement for GTP and could not be stimulated with salts. Crude cell membranes, on the other hand, were stimulated by salts. This suggested that crude membranes contained endogenous GTP. In order to study this and related problems further, we examined the action of the new GTP antagonist, GDP-β-S, to see whether GTP could be displaced from crude membranes and whether the salt effect could be antagonized. To our surprise, GDP-β-S, an antagonist or partial agonist in every other system studied proved to be a potent agonist for human AC, although it was also a GTP antagonist. Furthermore, the action of GDP-β-S was stimulated by salts. Accordingly, we studied the action of GDP in the human AC system. Current evidence in almost all AC systems indicates that GDP cannot substitute for GTP. However, in the human system, GDP proved to behave much like GDP-β-S. As a comparative extension of these observations we have studied GMP, a nucleotide with no GTP-like action for any other system. In the human AC system, however, GMP allows full expression of hormone activation. This startling finding calls into question the postulated mechanism of action of GTP in other systems. The findings are compatible with a permissive role for a guanine nucleotide, but are incompatible with the fashionable hypothesis that GTP inactivation occurs by hydrolysis of GTP to GDP. This work is being actively pursued and will soon be submitted for publication.

2) Changes in adenylate cyclase of the rat during starvation. "Tighter coupling" a new mechanism. An extension of the suspected role of diet in modulating age-related changes of lipolysis (5), led us to examine the mechanism of the increase of sensitivity of the lipolytic response to catecholamines that has been reported to occur during starvation.

Quantitation of β-receptors showed no change of receptor number or affinity as measured with antagonist, dihydroalprenolol (DHA). Similarly, the adenylate cyclase activity was not altered. However, the dose-response (hormone sensitivity) was increased 3-4 fold, (i.e., the dose-response curve was shifted to the left), indicating increased generation of cAMP at lower concentration of catecholamine. This phenomenon represents "tighter coupling" of the AC system. To our knowledge, such an alteration of the relationship between receptors and AC activity has never before been demonstrated. The finding of altered coupling under physiologic conditions represents a new kind of hormone regulation and deserves further study. We plan to explore the mechanism of this change in detail. This work has been accepted for publication (11).

3) Relationship of receptors to adenylate cyclase activity. In many systems the number of hormone receptors seems to be in excess of that needed for maximal hormone response (i.e., "spare" receptors). This relationship has not been defined for the adipocyte. Recently an irreversible β-adrenergic receptor antagonist has been synthesized. This compound has been used to reduce the number of β-receptors on rat adipocytes. Lipolysis is greatly reduced and the dose-response curve for catecholamines is shifted to the right. Further experiments should enable us to define the rat and human adipocyte receptor-cyclase interaction in precise and quantitative terms. Other questions which may be answered by such experiments relate to clarification of the interrelationships of the multiple receptors on the adipocyte. This work is in progress.

Significance to Biological Research and the Program of the Institute. The current studies are producing the basic information with which we can hope to understand the mechanisms of age-related changes of hormone responsiveness as they exist in the adenylate cyclase system. The interrelationships between receptors and adenylate cyclase activity and aspects of the biochemistry of adenylate cyclase continue to be explored.

Proposed Course of the Project: Further work on the human fat cell adenylate cyclase system will attempt to define its unique guanine nucleotide requirment, since this model may greatly alter our concepts of the mechanism of action of these regulatory compounds.

The mechanism of our newly discovered "tighter coupling" of the AC system will be explored. The first component which will be examined is the GTP-regulatory protein (G/F). Methods for quantitation of G/F are still being worked out in other laboratories, but our first attempts will involve ADP-ribosylation with labeled NAD and cholera toxin and a separate functional assay using adenylate cyclase from bull sperm, a complex which appears to be lacking G/F.

Diet manipulations other than acute starvation will also be examined for their effect on the AC system. Rats are being fed diets high and low in unsaturated fats. Others are being diet restricted. Plans are being made to study lipolysis in the Fisher rat, a strain which appears to differ in its age-related lipolytic responsiveness from the GRC Wistar we have used. This study should define whether diet or strain is responsible for the different age-changes we and others have described and will direct our further efforts in this area.

Publications:

1. Katz, M. S., Kelly, T. M., Piñeyro, M. A., and Gregerman, R. I.: Anions and cations as stimulators of liver adenylate cycase. Biochim. Biophys. Acta 632: 11-25, 1980.

2. Katz, M. S., Partilla, J. S., Piñeyro, M. A., and Gregerman, R. I.: Essential role of GTP in epinephrine stimulation of human fat cell adenylate cyclase. J. Lipid Res. 22: 113-121, 1981.

3. Pourmotabbed, G., Chou, H. J., Workman, R. J., and Gregerman, R. I.: Proteinase activity of human renin preparations: the International Reference Preparation (Renin Standard 68/356). Clin. Sci. 60: 633-637, 1981.

4. Pourmotabbed, G., Chou, H. J., and Gregerman, R. I.: A pepstatin-dextran conjugate as an inhibitor of proteinase-free human renin. Biochem. Pharmacol., in press.

5. Dax, E. M., Partilla, J. S., and Gregerman, R. I.: Mechanism of the age-related decrease of epinephrine-stimulated lipolysis in isolated rat adipocytes: β-adrenergic receptor binding, adenylate cyclase activity, and cyclic AMP accumulation. J. Lipid Res., in press.

6. Katz, M. S., Partilla, J. P., Piñeyro, Schneyer, C. R., and Gregerman, R. I.: Salts promote activation of fat cell adenylate cyclase by GTP. Special role for the Na^+ ion. Proc. Nat. Acad. Sci., in press.

7. Gregerman, R. I. and Bierman, E. L.: Aging and Hormones, Chapter 29. In Williams, R. H. (Ed.): Textbook of Endocrinology--ed. 6. Philadelphia, W. B. Saunders & Co., in press.

8. Gregerman, R. I.: The Thyroid Disorders, Chapter 70. In Barker, Burton and Zieve (Eds.): Principles of Ambulatory Medicine. Baltimore, Williams & Wilkins, in press.

9. Gregerman, R. I.: Diabetes Mellitus, Chapter 69. In Barker, Burton and Zieve (Eds.): Principles of Ambulatory Medicine. Baltimore, Williams & Wilkins, in press.

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11. Dax, E. M., Partilla, J. S., and Gregerman, R. I.: Increased sensitivity to epinephrine stimulated lioplysis during starvation: Tighter coupling of the adenylate cyclase system. Biochem. Biophys. Res. Commun., in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00012-09 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Hormones, hormone receptors and aging. II. Aging and hormone responsiveness.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	B. Baum	Dental Officer	LMA	NIA
	J. Rifkind	Research Chemist	LCMB	NIA
	P. D. Tsitouras	Visiting Associate (resigned 7/81)	CPB	NIA
	S. M. Harman	Senior Investigator	CPB	NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is mainly involved in relating age, the various components mediating hormone actions (including receptors) and the biological responsiveness of hormone sensitive tissues.

PHS-6040
(Rev. 2-81)

Objectives: This project attempts to elucidate the mechanisms by which the actions of hormones are altered during aging.

Methods Employed: Whole animals, isolated tissues, and defined cell populations in short term culture are used. Hormone receptors, located either on the cell surface or intracellularly, are studied qualitatively and quantitatively by measuring specific binding of labeled steroids, catecholamines and other hormones to tissues, cells and subcellular fractions. Affinity chromatography is used to isolate hormone receptors for measurement of synthetic and degradative rates as well as purification for preparation of antisera. Radioimmuno-and conventional assays are used for determination of hormone levels. Standard enzymatic, immunochemical and physiochemical techniques are employed. Hormonal control of various cellular metabolic processes such as nutrient transport and utilization are measured by standardized radiochemical techniques. Macromolecular biosynthetic processes are also assessed.

Major Findings: 1) Mechanisms of Steroid Action During Aging.

A) Glucocorticoid action in rat adipocytes. In addition to loss of glucocorticoid receptors, age-related decreases in glucocorticoid inhibition of glucose transport and metabolism appear to be due to generalized insensitivity of the adipocyte membrane glucose transport system to regulation. Further studies have optimized conditions for use of electron spin label probes to measure adipocyte membrane fluidity during aging. Protease inhibitors must be added to adipocyte ghost preparations to prevent degradation and artifactual increases in fluidity at 37°C. Preliminary data suggest that thermal melting curves are altered between maturity and senescence.

B) Estrogen action in uterus and skin. We have extended last year's observation that estrogen receptors are lost from rat uterus during aging by determining that such decreases are not due to increased nuclear localization, nor are they due to cell loss. Instead, reduced receptor concentrations appear most likely to be due to reduced biosynthethic rates, since no non-functional molecules can be detected immunochemically in aged tissue. Such age-related receptor losses correlate well with reduced ability of estrogens to regulate carbohydrate metabolism and cell proliferation as previously reported by others.

We have also observed an estrogenic potentiation of cutaneous wound healing in rats. Senescent animals require about 50% longer than mature counterparts to achieve half maximal healing. Since ovariectomy increases the time required for healing in mature but not senescent rats, either decreased estrogen levels or sensitivity may be involved in the age related reduction in healing rates. At present it is not possible to clearly distinguish between these two possibilities, although estrogen levels are low in most senescent animals and estrogen administration accelerates healing rates to within 20% of the mature group.

C) Permissive effects of glucocorticoids. Two hormone action systems exhibiting reduced responsiveness during aging are catecholamine stimulation of rat cardiac muscle contraction and gonadotrophin stimulation of Leydig cell steroidogenesis. In collaboration with the Cardiovascular Section we have confirmed suggestions in the literature that glucocorticoids are required for both responses. Moreover in the Leydig cell system, adrenalectomy has essentially no effect in senescent rats, while almost obliterating the response to gonadotropin in mature animals. Thus an age related reduction in sensitivity to glucocorticoids may be at least partially responsible for the impaired stereoidogenic response. We are currently measuring glucocorticoid receptors in both heart and Leydig cells as a function of age and adrenalectomy.

2) Mechanisms of Neurotransmitter Action During Aging.

A) Adrenergic/cholinergic effects in rat parotid cells and heart. As reported last year, we observed no age changes in the mechanisms (including β-adrenergic receptors) of β-adrenergic or cholinergic stimulation of amylase release or glucose oxidation in rat parotid cell aggregates. However, since older cells contain only about half as much amylase as young, absolute β-adrenergic and cholinergic release is decreased by half. In contrast, α-adrenergic stimulation of both glucose oxidation and potassium release are decreased with increasing age. The former response is reduced most markedly (∿50%) between 12 and 24 months of age, while the latter exhibits a 50% decrease between 3 and 12 months. Basal levels of glucose oxidation and potassium released are unchanged with age.

Selective agonist and antagonist studies revealed that parotid cells contain about equal portions of α_1 and α_2 receptors, although potassium release is mediated exclusively by the former. No loss of receptors or affinity was observed during aging. Instead, age changes in α-adrenergic stimulation of potassium release may be related to decreased stimulation of phospholipid metabolism and fluxes of calcium transport. Most interesting, if calcium concentrations are increased in the presence of the ionophore, A23187, cells from older animals release potassium to the same extent as those from young counterparts.

This last observation is particularly exciting since it is reminiscent of age related impairments in regulation of calcium transport which appear at least partially responsible for reduced β-adrenergic stimulation of rat cardiac muscle contraction. In an attempt to further elucidate possible membrane changes contributing to the age related alteration in adrenergic responsiveness we have established a system to measure phospholipid methylation, believed to be a possible second messenger for membrane active hormones, as a function of age in both the heart and parotid systems.

Since β-adrenergic mechanisms have been reported to be under thyroid hormonal control, we also examined the effects of thyroxine on cardiac hypertrophy and β-adrenergic receptors. Hypertrophy was maximal after 3 days of treatment and no age changes in time course or magnitude of the response were observed. β-adrenergic receptor levels increased about 100% after 7 days in mature rats. Although receptor levels in senescent hearts were not significantly different from those in mature organs after treatment, due to large inter-animals variability, the thyroxine effect was not significant in the older rats.

B) Dopaminergic mechanisms in the corpus striatum. Reduced levels of striatal dopamine receptors observed in aged mice, rats, rabbits and humans may be due to a decreased biosynthetic rate, as evidenced when receptor proliferation is induced chemically or mechanically. Such receptor loss may be related to decreased stimulation of dopamine sensitive adenylate cyclase and various dopaminergic behavioral effects.

We have attempted to further elucidate the mechanisms of dopamine receptor loss as well as precise identification of the receptor sub-types involved and possible mechanisms for their regulation. To this end we have established systems to measure the binding of the antagonists, haloperidol and spiroperidol, as well as the agonist, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN). The agonist appears to bind to a subset of the sites labeled by the antagonists and shows a larger age related reduction in binding. We are presently examining the effects of nutritional restriction and parabiosis of young/old pairs of animals on the age loss of various dopamine receptors.

C) Opiate receptors and sensitivity to pain. We have confirmed the reports of others for animals and humans that sensitivity to various painful stimuli decreases with age. However, such changes are apparently not the result of increases in rat CNS opiate receptor concentrations or affinities. In fact receptors are actually lost during aging in several brain regions. Alternative explanations for decreased pain sensitivity may be increased levels of endogenous opiates or increased release in older individuals.

Proposed Course of the Project:

1) A) Detailed analysis of the effects of rat age on adipocyte membrane fluidity properties will now be undertaken. Both protein and lipid electron spin labels will be employed.

B) We will attempt to further elucidate the mechanisms of altered estrogen action during aging in various tissues. In particular, emphasis will be placed on the role of circulating estrogens in controlling their own receptors and possible individual differences in receptor content of defined cell types during aging.

C) Further dissection of the molecular events controlled by glucocorticoids and their role in the age-related reductions in heart and leydig cell hormonal responsiveness will be attempted. In particular, glucocorticoid receptors will be measured in nuclei and cytoplasm in these tissues, both as a function of age and of adrenalectomy.

2) A) The control of phospholipid metabolism and calcium transport in parotid cells will be further examined. Selective inhibitors of these processes will be employed to attempt to establish causality for the age related alterations in alpha adrenergic responsiveness. Cholinergic agents may also be useful for this purpose since they stimulate the same calcium channels as do adrenergic agents, but different receptor mechanisms are utilized. It will also be important to determine why the loss of α-adrenergic responsiveness exhibits different age patterns for stimulation of potassium release and glucose oxidation.

The parotid cell system may also be useful for examination of other types of hormonal effects, including stimulation of DNA synthesis by catecholamines, which diminishes with age, but has never been demonstrated in vitro. It will also be important to examine phospholipid methylation in both the parotid and myocardial systems to determine to what extent alterations in this function may affect altered hormonal response during aging.

B) Examination of the effects of nutritional restriction and parabiosis with partners of different ages on striatal dopamine receptor loss will be continued. Ultimately, it will be necessary to prepare specific antisera against dopamine receptors so as to be able to determine whether age related losses are truly due to reduced biosynthesis, cell loss, or loss of receptor functionality, as we have done previously for steroid receptors.

C) We now wish to examine opiate receptors in other tissues and brain regions. Furthermore, it will be useful to examine opiate mediated responses in various systems so as to determine to what extent receptor loss during aging may affect tissue function.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00013-07 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors, and aging. III. Aging and human endocrine regulation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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SECTION
Endocrinology Section and Human Performance Section

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TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.3	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies gather data on function of pituitary gonadal and pituitary thyroid endocrine systems in normal aging men in the Baltimore Longitudinal Study on Aging (BLSA). Measurements of pituitary response to hypothalamic releasing factors and of metabolic processing of glycoprotein hormones as well as circulating levels of sex steroid hormones and thier relationship to sexual behavior in aging men are being undertaken.

Project Description:

Objectives:

A. Background - Steroid hormones secreted by the gonad play an important role in regulating body economy throughout the lifespan. In the male, testosterone, the major testicular steroid hormone, not only maintains secondary sex characteristics and sexual function, but is responsible for positive nitrogen balance and maintenance of increased muscle mass, for skeletal integrity, and possibly for such diverse functions as rate of healing and general level of aggressiveness. Steroid secretion by the testis is maintained by the pituitary hormone, LH, which is itself regulated by hypothalamic secretion of another hormone LHRH. This intricate control mechanism is in turn inhibited by rising plasma levels of testosterone, forming a complete system with its own feedback device to assure constancy of function. A second pituitary hormone, FSH, also under hypothalamic regulation by LHRH, controls testicular production of germ cells. LH, FSH, (and TSH) are glycoprotein hormones consisting of two subunits, a common α subunit and a β subunit which confers hormonal specificity on the molecule. The pituitary gland also produces prolactin, the hormone responsible for induction of lactation in women. The secretion of prolactin is normally repressed by a hypothalamic inhibitory factor and can be stimulated by hypothalamic TRH, the same substance which is responsible for pituitary release of TSH. This latter hormone, the thyrotropic hormone, stimulates thyroid incorporation of iodine into thyroxine, secretion of thyroid hormones into the blood and growth of thyroid tissue. Prolactin is known to inhibit gonadal function in the male both by decreasing pituitary release of gonadotropins and by some direct interference with testicular production of androgens. There also is an increased incidence of impotence in men with elevated prolactin.

B. Current knowledge - Previous investigators have described a decrease in the circulating level of testosterone after age 60, an increase in plasma protein binding of testosterone, which further lowers the level of "free" (and thus, presumably, bioavailable) testosterone, and an increase in circulating levels of female hormones (estrone and estradiol). At the same time the plasma FSH and LH levels have been found to increase, suggesting a primary failure of the testis, which releases the pituitary from feedback inhibition control. Some evidence also exists for a reduction in pituitary function with age in that response of the pituitary to exogenous LHRH appears to be somewhat reduced. Various investigators have found elevations of free α subunit in older humans but whether this represents unbalanced synthesis of α and β chains (i.e., loss of efficiency) is not clear. Since study populations have not been well-defined in terms of variables which may affect hormone secretion such as nutrition and obesity, alcohol and tobacco consumption, and health and general level of activity, it is difficult to interpret the significance of this data to aging, per se. Furthermore, none of the available studies has a longitudinal design, which makes their interpretation subject to all of the difficulties which characterize cross-sectional aging studies. We have recently published results showing that in BLS men there are no changes in sex steroid hormones from age 25 to 89. Nonetheless, there does seem to be a rise in LH and FSH. Furthermore in a follow-up study in 180 men (paper submitted) we found a correlation of sexual frequency and testosterone in men over 60 which was not mediated by body type, smoking, alcohol consumption, or the incidence of coronary artery disease.

A number of studies have suggested that thyroid failure with low thyroxine and elevated TSH may be more common in elderly people. Pituitary release of TSH in response to TRH also seem impaired with age according to some sources. A preliminary report has described an increase in prolactin in aging men.

C. Present study - Using a well-characterized group of men from the Baltimore Longitudinal Study on Aging, the effects of age on testicular function and pituitary-gonadal regulation independent of illness, excess alcohol consumption, obesity, etc. are being defined. In addition, we are now studying effects of age on pituitary-thyroid dynamics. Additional studies to improve techniques of glycoprotein hormone assays and to determine the rate at which old vs. young humans clear these hormones from the circulation are also underway.

Methods employed: (1) Plasma gonadotrophins prolactin and TSH are assayed using double antibody radioimmunoassays. (2) Plasma testosterone and dihydrotestosterone are measured with an improved radioimmunoassay using Florisil separation. (3) Plasma estrone and estradiol are assayed using a charcoal radioimmunoassay method. (4) Plasma thyroxine and triiodothyronine will be assayed using routine radioimmunoassay methods. (5) The fraction of free testosterone in plasma is estimated by an ion-exchange column method developed in our laboratory. (6) Blood samples are obtained from a healthy, non-obese subgroup of the BLS population before and after constant intravenous infusion of 96 µg of TRH over 4 hours to test for pituitary TSH secretory reserve. (7) Computerized techniques have been developed to evaluate data. (8) Subjects will be restudied at 8-10 year intervals to provide longitudinal data for both LHRH and TRH stimulation response alternating these two tests.

Major Findings:

A. Effects of aging in men on pituitary responsiveness to gonadotropin releasing hormone (GnRH). 1) In collaboration with Dr. Paul Costa of the Laboratory of Behavioral Sciences, a sophisticated statistical approach (repeated measures ANOVA and stepwise discriminate analysis) has been devised for comparing pituitary responses (secretion of gonadotropic hormones, LH and FSH) to GnRH in groups of experimental subjects whose baseline levels of gonadotropins differ. Using this approach we have shown that a) GnRH stimulation has discriminative value in diagnosing hypopituitary disease and b) that the pituitary reserve for gonadotropin secretion is relatively diminished in older participants in the BLSA. A manuscript describing the value of this technique in diagnosis has been prepared and submitted and a revised manuscript, reporting its application to the aging population, is in preparation 2) Analysis of blood samples obtained before and after GnRH stimulation of BLSA men for the free subunits of LH and FSH has shown a decrease with age in the ratio of the common α subunit to intact gonadotropins in baseline sera but a relatively greater amount of α subunit secreted by older men after GnRH stimulation (although the absolute amount of α secreted is less in older men). There is a decrease both in the absolute amount of the specific β subunits of LH and FSH secreted and in the LHβ/LH and FSHβ/FSH ratios after GnRH stimulation. These data suggest that β subunit synthesis (and not α) is the limiting step in gonadotropin secretion in aging men and that increased gonadotropin secretion may be obtained at the expense of synthesis of a greater excess of α subunit so that a greater percentage of β subunits produced will be utilized. This study points the way to further analyses of the kinetics of protein hormone syntheses, assembly, and secretion in aging humans and other mammalian models.

B. Metabolism of the gonadotropin, LH, in different phases of the menstrual cycle and during administration of an estrogen-progestin combination pill. In order to study the metabolic disposal of gonadotropins in aging humans, using a constant infusion technique, it will be necessary to suppress the high endogenous production rate seen in post menopausal women. In order to determine whether there is an independent effect of administering suppressive doses of estrogen and progestin on metabolic disposal of LH, 5 young women were studied during days 4-12 (follicular) and also days 20-24 (luteal) of their menstrual cycles and again after 8-12 days administration of an estrogen progestin combination. Results show a remarkably constant rate of LH disposal for each individual with larger between subject variation. Paired t-tests revealed a small but significant increase in LH disposal rate in the luteal phase of the cycle, but no significant effect of estrogen-progestin administration on LH metabolic clearance. This study clears the way for similar studies in elderly men and women. Data on rates of disposal of hormones in this population is needed to complete our understanding of altered glycoprotein hormone kinetics with age.

C. Use of an antibody against the unique c-terminal peptide portion of the human chorionic gonadotropin (hCG) molecule with special techniques of concentration and purification to improve sensitivity and specificity of detection of hCG in urine. The hCG molecule is produced by trophoblastic tissue during pregnancy, by trophoblastic and germ cell neoplasms, and with some frequency by various other malignant neoplasms. Detection of hCG is complicated by its antigenic and bioactivity resemblance to pituitary LH. Use of an antibody against a unique peptide, found in the beta subunit of hCG but not in LH (c-terminal peptide = CTP) has enabled us to detect very low levels of hCG with the ubiguitous LH molecule. Kaolin acetone extraction following by concentration of glycoprotein on a lectin-gel (concanavalin A-Sepharose) make it possible to conveniently assay an entire day's urinary output of hCG as a single sample. This technique has been demonstrated to discriminate patients with residual trophobalstic tumor from patients who have been "cured" when the usual serum assay for hCG is negative. It holds promise as an investigative tool to detect very small, early hCG secreting tumors and also to determine whether aging is associated with persistence or establishment of abnormal clones of cells making hCG or its β subunit.

Proposed Course:

A. Evaluation of pituitary thyroid axis and prolactin secretion and reevaluation of pituitary gonadal axis on BLSA men. This study, currently underway, will produce data on the function of the pituitary thyrotroph and lactotroph in normal aging men. Briefly, BLSA men screened for health and drug use are given a 4 hour low dose constant infusion of TRH, the thyrotropin releasing hormone, which stimulates release of TSH (thyrotropin) and also prolactin. These hormones and also the α and TSH β subunits are being measured in all sera. In addition, thyroid hormones, serum sex steroids, and gonadotropins are being determined in basal sera of this cohort which will include nearly all the men studied in our previous investigation. Thus, we will begin to build up a longitudinal profile of pituitary gonadal and pituitary thyroid physiology in the BLSA. Approximately 40 subjects have been studied up to July 1, 1981.

B. Effects of aging on glycoprotein hormone metabolism. This study will help to determine whether the increased LH and FSH levels seen in aged men and women may occur in part due to reduced rates of glycoprotein metabolic disposal. Infusion of purified human LH to constant serum levels, analysis of urinary disposal rates, and identification to metabolic products of LH in blood and urine by gel filtration chromatography will help to elucidate this issue. Additional studies on the relative ratio of immunoreactive to bioactive (receptor assay and ability to stimualte T production in Leydig cell suspensions) native LH from blood of young and old subjects will also be undertaken and will contribute to our understanding of the alterations of these important messenger molecules with age.

C. Comparison of effects of age and disease on serum T and gonadotropin levels. A large collection of sera from patients of various ages with various malignant and non-malignant diseases will be analyzed and compared with sera from normal young and old BLSA subjects to determine whether our contention that illness may account for the decreased T reported in previous aging studies is sustainable.

D. Diurnal variations of T and gonadotropins in aging males. An early morning increase in T with return to baseline levels by about noon has been described in normal young men. A preliminary report at the Endocrine Society (1981) meeting suggests that this diurnal rhythm may be absent in older men. We plan to use constant withdrawal pumps to get integrated half hourly blood samples from ambulatory volunteers over a 24 hour period in order to investigate this issue in BLSA men.

Significance to Biomedical Research and the Program of the Institute. The studies described and proposed not only add to the fund of normative data on aging but also illustrate the necessity of a sophisticated design and statistical approach to experiments on aging humans. Furthermore, they go beyond description in attempting to elucidate changes seen with age in terms of responsible physiologic mechanisms (e.g. protein hormone synthesis and assembly of subunits). Therefore they should help point the way toward a deeper scientific understanding of the nature of aging changes in human metabolism.

Publications:

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Harman, S. M., Tsitouras, P. D., Costa, P. T., Sherins, R., and Loriaux, D. L.: Evaluation of pituitary gonadotropic function in men: Values of LHR response vs. basal LH level for discrimination of diagnosis. J. Clin. Endocrinol. Metab., in press.

Harman, S. M., Tsitouras, P. D., Costa, P. T., and Blackman, M. R.: Reproductive hormones in aging men. II: Measurement of basal gonadotropins and gonadotropin response to gonadotropin releasing hormone. J. Clin. Endocrinol. Metab., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00023-06 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors, and aging. Testis, pituitary, and hypothalamic function in aging and uremia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	G. S. Roth	Senior Investigator	CPB	NIA

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TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.2	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Secretory cells from rat gonads and pituitaries are studied in vitro to compare their physiology in old and young rats and in normal vs. uremic rats. Testosterone production, in response to gonadotropin and to dibutyryl cAMP and cell number of gonadotrophin receptors, are measured in order to investigate alterations of Leydig cell function. Production of TSH in response to TRH, production of LH and FSH and their subunits in response to LRH and receptors for LRH have been measured in order to investigate altered function of pituitary secretory cells. Hypothalamic content of LRH, and turnover rates of dopamine and catecholamines in hypothalamic nuclei are being determined. Deficient function of aged pituitary cells in vitro has been found as has a reduction in LRH receptor affinity. Castration increases LRH responsiveness of gonadotrophs of aged and young rats to the same extent.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The Leydig cells of the testis secrete testosterone, a hormone essential to male reproductive development and function, which also has important actions at sites as diverse as bone, muscle, skin, and central nervous system. A number of studies have shown reduced function of Leydig cells with age both in animals and in man. Leydig cell function is normally under the control of the pituitary hormone, LH, which is secreted under the influence of the hypothalamic releasing factor, LRH. LH interacts with Leydig cell receptors to activate the enzyme adenylate cyclase. The resultant increase in cAMP leads to catalytic phosphorylation and activation of a system of protein kinases, which in turn alter the cell's internal metabolism and lead to hormone production and secretion. One of the goals of the present study is to define and investigate the nature of the defect appearing in an animal Leydig cell system with advancing age. Previous findings have shown that a significant minority of older rats studied were uremic and uremia is known to inhibit gonadal function. Furthermore, studies in our own and other laboratories have suggested that the Leydig cell deficiency in older rats is at least in part due to reduction in LH activity and thus may be a result of altered hypothalamic-pituitary function. Finally it is known that 1) normal adrenal function is necessary for optimal secretion of testosterone and that Leydig cells contain glucocorticoid receptors, and 2) that a reduction in these receptors occurs in various types of cells in old animals. Therefore we have expanded our studies to investigate the effects of uremia and of adrenal hormones on Leydig cell function and of aging and uremia on pituitary cell physiology and hypothalamic biochemistry.

Methods Employed: Matched pairs of young (4-9 months) and old (22-26 months) rats from the GRC Wistar colony are killed by decapitation and the testis removed and partially digested with collagenase. In uremia studies young animals are made uremic by partial nephrectomy and studied with paired sham-operated rats after 6 weeks of uremia. Tubular and interstitial elements are separated by filtration. The number of viable Leydig cells in each preparation is estimated using a histochemical (3-β-hydroxy-dehydrogenase) stain and the trypan blue exclusion technique. Short term incubations are then carried out with varying doses of human chorionic gonadotrophin (hCG, an LH-like hormone) or Dibutyryl cyclic AMP to determine cell production of testosterone. Cell membrane binding capacity and affinity for radioactively labelled hCG is determined to estimate receptor number and quality. Testosterone is analyzed by radioimmunoassay using Florisil for separation of bound and free hormone. Pituitaries are removed, pooled, digested with collagenase, and after 24 hours of preincubation cell function is assessed by adding various doses of stimulating substances (LRH, TRH, dibutyryl cAMP, etc.) to the media and measuring secretory products (LH, FSH, TSH, and α, LHβ, FSHβ, and TSHβ subunits) released. Hypothalami are removed, homogenized, and aliquots set aside for determination of LRH by radioimmunoassay. Turnover rates of catecholamines and dopamine in various nuclei of the ventral hypothalamus are estimated by a technique using paramethyltyrosine to block synthesis of these neurotransmitters, then doing microdissections of various brain nuclei and determining rates of depletion of transmitter using sensitive radioenzymatic assays.

Major Findings: Continued experiments have confirmed our preliminary results that the "aging" defect is reversible with hCG treatment in vivo, and that serum FSH and LH are reduced in aged rats. HCG treatment improves performance of old cells despite 85 to 90% reduction in available membrane receptors for hCG in both young and old cells. Similar levels of cAMP are found in young and old cells. These findings suggest that the "aging defect" in old rats may be mainly an effect of gonadotropin deficiency rather than an age related alteration of intrinsic Leydig cell metabolism. Work showing reduced sensitivity of aged cells to adrenal corticosteroid and the prior knowledge that deprivation of adrenal steroid stimulation leads to gonadal hyposecretion of testosterone (the major male hormone) led us to conduct experiments on the effect of adrenalectomy on function of Leydig (secretory) cells isolated from testes of young and aged rats. Leydig cells from adrenalectomized young rats are hyporesponsive to hCG compared with young intact rats, but adrenalectomy does not seem to affect the (already impaired) function of aged cells any further. This finding is consistent with either insensitivity to or deficiency of adrenal hormone in aging rats. Further investigations are underway to make this distinction.

Repeated experiments have also agreed that one or more of the enzymes in the steroid synthetic pathway proximal to the final step may be deficient in activity and that the final enzyme in the pathway (17α keto reductase) is deficient. Our study of uremic rats has demonstrated reduction in testosterone secretion similar to that seen in aged cells, but uremic cells differ in that they improve their response to hCG with continued exposure in vitro, while aged cells do not, and uremic cells appear to have significantly fewer hCG receptors than aged cells.

Experiments with pituitary cell suspensions have demonstrated reduced secretion of LH, FSH, and α subunit by cells isolated from old rats when stimulated with LRH. Although performance of both old and young improved after castration, the difference appears to remain constant even 3 weeks post-castration. This suggests that performance of old pituitaries is not normalized by increasing endogenous LRH secretion in vivo. The reduction in glycoprotein secretion is unbalanced in that the α subunit to intact hormone ratio is decreased when cells from older rats are employed.

Our assay for hypothalamic LRH indicates that total hypothalamic content of LRH is unchanged by uremia and also remains constant with aging. Preliminary efforts to measure the binding affinity and capacity of pituitary cell membranes for LRH have suggested that there may be a reduced binding affinity of the LRH receptor from old pituitaries but no loss of binding capacity. This suggests either an alteration of membrane proteins, or of the physical state of the membrane itself and could be partially responsible for the observed reduction in LRH response. Measurements of the concentrations and turnover rates of neurotransmitters (dopamine and norepinephrine) in the various hypothalamic nuclei which regulate gonadotropic function show significantly reduced levels of norepinephrine in the arcuate nucleus and also reduced rate of depletion with time (presumably reflecting decreased nerve activity) in the arcuate and paraventicular nuclei. Further work will be necessary to elucidate the nature of these changes and their relationship to decreased gonadal function in aged rats.

Significance to Biomedical Research and the Program of the Institute: If the nature of aging is to be understood, the precise biochemical defects in the function of differentiated cells of aging animals as well as the defects hindering the replacement of such cells from populations of less differentiated cells must be investigated. Since the characteristic response pattern and details of many of the intermediate steps in the metabolism of Leydig cells is known, and since secretory and synthetic mechanisms of pituitary and hypothalamic neurosecretory cells are being worked out, these models seem to be well suited for the study of aging processes, both on a cellular basis and from the point of view of an interacting multicomponent homeostatic system.

Proposed Course: Performance of old and young Leydig cells will continue to be evaluated in the presence and absence of various steroid precursors, cofactors, and energy sources. Rate limiting enzyme activities will be assayed. Comparison of uremia and aging will continue. More emphasis will be placed on working out the physiology and biochemistry of aging pituitaries and hypothalami with further determinations of receptors, hormones, and neurotransmitters and expansion of this latter work to include the pituitary thyroid axis and pathways of synthesis and assembly of glycoprotein hormones. In collaboration with Dr. S. Humphries we will attempt to identify pituitary cell types using immuno-histochemistry at the electron microscope level and to quantitate different cell types in aging pituitaries.

Publications:

Mezey, E., Potter, J. J., Harman, S. M. and Tsitouras, P. D. Effect of castration and testosterone administration on liver alcohol dehydrogenase activity. Biochem. Parmacol. 29:3175, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00015-23 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Baltimore Longitudinal Study of Human Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
	J. D. Tobin	Chief, Human Performance Section	CPB NIA
	N. W. Shock	Scientist Emeritus	NIA
	S. K. Sharma	Sociologist	CPB NIA
	A. H. Norris	Former Chief, Human Performance Section	CPB NIA
OTHER:	L. Brant	Mathematical Statistician	CPB,NIA

Other workers who are associated with the Longitudinal Study

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 12.36	PROFESSIONAL: 4.06	OTHER: 8.30

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Baltimore Longitudinal Study of Human Aging (BLSA) serves as a resource for scientists working in the field of Gerontology. It provides a well-described group of men and women between 20 and 96 years of age for studies of the mechanisms of human aging. Projects in physiology, biochemistry, psychology, nutrition, pharmacology, endocrinology, sociology, and genetics, have been carried out or are in progress.

PHS-6040
(Rev. 2-81)

Project Description: Z01 AG 00015-23 CPB

Objectives: The BLSA provides a well described group of subjects as a resource in support of a wide variety of scientific investigations in gerontology and other disciplines. While long-term planning is encouraged, important studies of shorter duration have also been undertaken. The long-term general goals of the project are to: (1) secure replicate measures of physiological, pathological, biochemical and psychological variables on longitudinal study participants at specified intervals; (2) summarize and compare the results of testing in relation to age according to cross-sectional and longitudinal formats; (3) identify characteristics of individual participants which may be related to changes of function over time and to age at death; and, (4) determine whether the data obtained support one or another theory of the mechanisms responsible for age-related functional decrements.

Methods Employed: The Sample: Study participants are male and female volunteers recruited by other participants in the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing every 12 months (age 70 and over), 18 months (age 60-69) or 24 months (under age 60) for an indeterminate period. Our sample continued to be highly educated, mostly married, describing themselves as financially comfortable or better, and of the group who returned for the fifth visit, 90% rated their health as good or excellent on both first and fifth visits. The age distribution of the active subjects is shown in Table 1.

Table 1: Distribution of the Active Subjects by Age and Sex (in percent).

Age Group	Sex	
	Men	Women
> 20	0.2	0.0
20-29	3.1	5.9
30-39	19.4	22.2
40-49	13.8	16.7
50-59	20.5	18.9
60-69	21.1	23.7
70-79	13.6	11.5
80-89	7.5	0.7
90+	0.8	0.4

Data Management: Medical records and test results are maintained in written form in the laboratory and transferred to a data retrieval and analysis system by keypunching on tabulation cards or by recording the test results directly on punched paper tape or magnetic tape. Data are maintained and used in ways which protect the privacy of participants. Sensitive material is specially encoded. Individual scientists review, evaluate and summarize the data for scientific reporting.

Major Findings: By August 12, 1981 a total of 1147 men and 296 women have participated in the testing program on one or more visits to the GRC. Since the inception of the study 255 men have died. As of August 12, 1981, 884 men have completed three or more visits for testing, 668 visited five times or more, 302 ten times or more, 165 twelve times or more, and

46 fifteen times or more. In all, these subjects account for a grand total of 7417 participant visits.

During the year 49 women were newly admitted to the program as compared to 10 men. Of the 296 women who have joined the BLSA since January 1978, three have died and 7 withdrew leaving an active sample of 286. One hundred forty-four women have been tested two times; 35 have been tested three times or more.

The Illness and Disability Study (I&D) continues to be an integral part of the BLSA.

The primary objectives of the I & D Study continue to be:

1. To locate, contact and gather information pertaining to the subsequent health and illness experiences of those subjects who dropped out from the BLSA between February 6, 1958 to July 1, 1977.

2. To ascertain the illness and disability experiences of drop-outs subsequent to their last visit to the BLSA until present and to compare it with the illness and disability experiences of those who remained active in the study.

3. To determine whether subjects who dropped out from the BLSA differ significantly from subjects who remained active in the study with regard to: a) demographic variables, b) psychological variables, and c) physiological variables, as measured at the time of first visit to the BLSA.

4. To ascertain the risk factors for dropping out from the BLSA and to recommend some control measures aimed at reducing the dropout rate.

As of July 1, 1977, there were 1088 subject who had made one or more visits to the BLSA since the inception of the study in February 1958. Of those 1088, six hundred and fifty-eight (60.5%) were still active in the study. One hundred and fifty-seven (14.4%) failed to return to the BLSA. Ninety-six (8.8%) formally withdrew from the BLSA. The remaining one hundred and seventy-seven (16.3%) were known to be dead as of July 1, 1977. (Table 2)

Table 2: Frequency Distribution of BLSA Subjects by Status
N = 1088 (July 1, 1977)

Status	No.	Percent
Active	659	60.48
Failure	157	14.43
Withdrawn	95	8.82
Deceased	177	16.27
	1088	100.00
Total attrition in 20 years by reason other than death		23.25%
Annual attrition rate		1.16%

Methods: First, an attempt is made to locate all the drop-outs (those who have failed to return for tests and those who have formally withdrawn). After the subjects are located, a letter is mailed to the subjects explaining this study and requesting their cooperation. This letter is followed by a telephone call approximately 10 days after the letter is mailed. Subjects are encouraged to return to the BLSA, for one or more visits. If the subject can not come to the BLSA for any reason, an attempt is made to get their consent for a home visit by the investigator. If a home visit is not possible (due to distance), an attempt is made to get consent for an Interval Medical History by mailed questionnaire. In addition, these subjects are asked to provide consent for the investigator to obtain information from any physician/s and hospitals that they may have visited during this interval period. If the subject refuses the questionnaire, an attempt is made to get consent for a telephone interview.

In the beginning of the study, it was decided to begin first with subjects who had failed to return to the BLSA (N=157). The first phase of the study was to locate and contact the subjects who had failed to return. The location rate was 100%. The response rate was 98%. Thirteen subjects were found to be dead subsequent to their last visit to the BLSA. The response rate of the alive subjects is given in Table 3.

Table 3. Response Rate of Failed to Return Subjects in Illness and Disability Study.

N = 157

Alive	Dead
n=144	n=13
91.7%	8.3%

Response	N	Percent
Returned to BLSA	60	41.7
Home Visit	22	15.3
Questionnaire	46	31.9
Telephone Interview	6	4.2
Undecided	7	4.9
Unable to Contact	2	1.4
Dropped from Study	1	.7

Of these 157 subjects, 13 subjects (8.3% were found dead at the time of first contact. Information with regard to data of death and cause of death has been obtained on these deceased subjects.

The second phase of the Illness and Disability Study was to locate and contact the subjects who had formally withdrawn from the BLSA and were known to be alive as of July 1, 1977 (N=95). Our methods were the same as in the case of subjects who had failed to return to the BLSA. The second phase began in August 1980 and the response rate so far is given in Table 4.

Table 4. Response Rate of Withdrawn Subjects in Illness & Disability Study.

N = 95

Found Alive	Found Dead
N = 81	N = 14
85.3%	14.7%

Response	N	Percent
Returned	10	12.3
Have definite appointment	3	3.7
Home Visit	17	23.0
Questionnaire	21	25.9
Telephone Interview	3	3.7
Undecided	15	18.5
Yet to locate	11	13.6
Refused to cooperate	1	1.2

Suceeding phases of the I & D Study will include:

1. Data with regard to health, illness and disability experiences of subjects who failed to return and withdrew will be analyzed.

2. We plan to use a non-parametric survivorship analysis to generate and compare survivorship pattern for each group (i.e. those with or without a characteristic). Kaplan-Meier product-limit estimates will be used in generating a survivorship curve for each of the groups. The two survivorship curves will then be compared using the Mantal-Cox and Breslow tests of significance.

In addition, we plan to study the association between a number of factors believed to be related to survivorship in the BLSA. The methods purposed are: (a) multiple logistic regression and (b) binary variable multiple linear regression. For factors found to be significant, we plan to estimate the relative contribution of each factor, while controlling for the effects of other factors.

Findings of this study will update the BLSA records. The validity of the data already obtained and the generating of the research findings would be enhanced. The identification of risk factors for dropping out of the study may be used to facilitate the development of control measures aimed at reducing the dropout rate in the BLSA in the future.

Significance to Bio-Medical Research and the Program of the Institute: A major goal of the longitudinal program is a deeper understanding of age-related changes in the different organ systems, and their interrelationships. The relation of functional changes in an individual to age at death, age at onset of a disease, and other end points is important for understanding aging in humans and the impact of aging on society. The intensive study of multiple variables will also provide tests of risk-factor theories for specific age-related diseases.

Proposed Course: Data collection and analyses will be continued. Continued emphasis on automation of tests, data entry, and analyses should provide improved accuracy and efficiency. A major summary of all aspects of this program is in progress.

Publications:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00018-15 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Marital, Sexual and Social Factors in Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Martin	Sociologist	CPB NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0.80	PROFESSIONAL: .70	OTHER: .10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Systematic data relating to marital, sexual, and social experience were obtained by means of structured interviews with male members of the Baltimore Longitudinal Study on Aging. During the year stepwise multiple regression procedures were used to identify those factors which, alone or in combination, best predicted current frequency of sexual expression. The best three-variable model which emerged included: time comfortable without sex, age at report, and customary frequency of coitus in the first year of marriage. Why these particular variables proved to have a predictive function and how they may be useful in future inquiry are questions of some importance.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Study objectives continue to be: 1) to pursue the question as to why some males are more, or less, active sexually than others, 2) to determine whether one or another kind of marital or sexual history may be related to certain entities of disease or disability and 3) to interview the few male members of the longitudinal program who have not as yet completed interviews.

Methods Employed: In the course of his participation in the study, each longitudinal study member is asked to contribute an interview concerning his history of marriage and sexual activity. In making this request, the author delineates study objectives, provides assurance of confidence and emphasizes the voluntary nature of such a contribution. Over the years the refusal rate has varied from 2 to 3 percent.

To facilitate communication and help insure systematic data collection, the questions asked were memorized by the investigator along with whatever categories were required for the classification of responses. To aid memory, establish rapport and generate data not otherwise obtained, a number of aspects of occupational, educational, religious, military and parental-home experience were reviewed before introducing questions pertaining to marital adjustment and sexual conduct. Because of the excellent mental status of respondents and their evident interest in this part of the testing program, the information obtained is believed to be of exceptional quality.

Major Findings: Earlier inquiry into the nature of male sexual motivation had revealed some seven factors which were significantly related to frequency of sexual expression (activities conducive to orgasm) in 60-79 year old respondents. Of considerable interest was the close statistical interelationships found between the three variables: sexual frequency, time comfortable without sex, and erotic reactions from seeing female nudity. This interaction, along with the observation that males commonly experience a marked reduction in sexual interest when confined to all-male social environments, indicated that, in the male, erotic reactions to visual stimuli play a key role in sustaining feelings of sexual need. This interpretation also suggested that these variables are casually connected as follows: erotic reactions to visual stimuli → time comfortable without sex → sexual frequency.

Further inquiry, based upon the entire sample of 24-79 year old male respondents, took the form of a stepwise multiple regression analysis of the seven factors involved to determine which factor or combination of factors might best account for individual differences in sexual frequency. The variables employed were: 1) erotic reactions from seeing sexual nudity, 2) time comfortable without sex, 3) age at interview, 4) potency status, 5) customary frequency of coitus in the first year of marriage, 6) maximum number of coital events in any single week of marriage, and 7) quantity of total sexual activity recalled for ages 20-39. In following table, R^2 describes that percent of variance which is explained by each factor or combination of factors. As will be seen, the best three-variable model identified by the procedure included: time comfortable without sex (32%

of variance), age at interview (28% of variance), and customary frequency of coitus in the first year of marriage (11% of variance), whereas together these three variables accounted for 45 percent of total variance.

Variable No.	N	Multiple R	R^2
1	659	.30	.09
2	619	.57	.32
3	667	.53	.28
4	664	.36	.13
5	665	.34	.11
6	664	.30	.09
7	528	.41	.17
Best two-variable model			
2,3	621	.63	.40
Best two-variable model, excluding age			
2,5	619	.61	.37
Best three-variable model			
2,3,5	619	.67	.45
Split-half comparison (three-variable model)			
Even subjects, Odd B values			
2,3,5	307	.66	.44
Odd subjects, Even B values			
2,3,5	312	.68	.46

Significance to Bio-medical Research and the Program of the Institute: The human organism is, in many respects, ideally suited to the study of sexual motivation because its various subjective aspects as well as its overtmanifestations are readily reportable. That 'time comfortable without sex' should prove to be a better predictor of sexual frequency than age is testimony to its importance as a direct measure of current motivation-a measure that may have a wider usefulness as a dependent variable in further analysis.

With respect to the factor of age Pfieffer & Davis (J. Am. Geriat. Soc. 20: 151, 1972) in a similar multiple regression analysis pertaining to coital frequency, found that age explained 26 percent of total variance, comparable to the present 28 percent figure. Age, however, will continue to be of limited usefulness until more is learned about the nature of the interaction of age and sexual functioning.

Finally, the emergence of 'customary frequency of coitus in the first year of marriage' as an important predictor of current rate of sexual activity, while not wholly unexpected, appears remarkable considering the wide range of ages of the men involved. On the other hand, for most respondents early marriage was a period of intense erotic interest and heightened rates of activity, so that the level of activity established at the time was fairly representative of the males ability to perform. In any event, coital frequency in early marriage, as determined by retrospective inquiry, has proved to be a most consistent correlate of every other measure obtained in the study pertaining to quantity of sexual expression.

Proposed Course: The major objective for the year is to continue data analysis and the preparation of manuscript. Much of the analysis will be multi-variant in character, incorporating a range of physiologic and psychologic variables, in an effort to improve our understanding of the nature of male sexual motivation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00021-18 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Study of Normal Human Variance: 1. Dermatoglyphics 2. Lateral Dominance 3. Visual Function 4. Color Vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
See attached page.

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.64	0.54	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project represents A) an ongoing joint collaborative effort, involving the WHO and other national and international biological laboratories to coordinate the collection, evaluation and interpretation of dermatoglyphic data. Specifically the objectives of this project are: 1) to establish dermatoglyphic markers in various diseases (clinical dermatoglyphics); 2) to establish the dermatoglyphic frequencies in normal control samples (control dermatoglyphics); 3) to study the distribution of dermatoglyphics among the various human populations (population dermatoglyphics); 4) to study the dermatoglyphics of the aged: 5) to study the genetics of dermatoglyphics; and, 6) to utilize dermatoglyphics as an added tool in twin diagnoses (twin dermatoglyphics). B) Evaluation of lateral functional dominance among BLSA participants. C) Cross sectional and longitudinal study of visual function in BLSA participants.

PHS-6040
(Rev. 2-81)

Cooperating Units: Z01 AG 00021-18 CPB

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Project Description:

Objectives: A) the ongoing dermatoglyphics project represents an extensive collaborative effort in conjunction with WHO and other national and international institutions, to study all aspects of dermatoglyphics. The specific objectives of this study are: 1) To utilize dermatoglyphic markers in clinical investigations, 2) To establish dermatoglyphic frequencies in non-affected, control samples from different human populations, 3) To utilize dermatoglyphic markers in studies of population genetics, twin diagnosis and other genetic investigations. 4) To determine possible effects of chemical pollutants on embryonic development through dermatoglyphic anomalies. B) To establish the hand, foot and visual lateral dominance in BLSA participants in an effort to understand the normal physiological and anatomical bilateral asymmetry. C) to establish cross sectional age specific visual function levels in BLSA participants and investigate through longitudinal studies the changes in visual function.

Methods: Digital and palmar prints collected by different groups through various methods are sent to our laboratory for evaluation and interpretation of the results. We developed new methods and computer programs for studying and analyzing the dermatoglyphic data. These methods have been accepted and are utilized by other laboratories here and abroad.

In order to determine lateral functional dominance the participants were requested to perform seventeen tasks. Two of these relate to single hand involvement; two tests relate to single hand fine manipulation; three tasks involve fine function of one hand with significant assistance from the subordinate hand; three tasks are single handed activities which involve whole body movement. In addition to these handedness tests, the participants were tested for foot dominance, eye dominance (Dolman test), digital interlocking, arm folding and foot overlapping. At the completion of these tests the participant is asked whether he considers himself/herself as left handed, right handed or ambidextrous. Many participants were tested in more than one visit to determine repeatability of the tests.

Vision tests are administered with a Titmus optical vision tester which simulates vision at both 20 feet and 14 inches. Acuity is measured with both Sloan letters and Landolt Rings. Depth perception and vertical and lateral phorias are assessed. The subject is tested with glasses, if he or she wears them as well as without glasses. Color discrimination is tested with the American Optical Pseudo Isochromatic Plates.

Major Findings: A. Dermatoglyphic comparisons showed that Guamanian patients with either Amyotrophic Lateral Sclerosis (ALS) or Parkinsonism Dementia (PD) have significantly higher frequencies of complete simian creases than control nonaffected Guamanians. In addition ALS patients had significantly higher frequencies of palmar patterns and accessory triradii in the IV interdigital area than controls. No significant differences were observed in the comparisons between the two patient samples. B. Differences between ulnar and radial counts of loops and whorls suggested that lateral evaluation of ridge counts may provide additional polymorphic dermatoglyphic markers which could be utilized in clinical, genetic and anthropological studies. C. Bilateral measurements of the second metacarpal bone of left and right handed participants, suggest that there is an inherent tendency for larger bone in the right side, regardless of lateral dominance (determined through grip strength). Right hand dominance will increase this difference by enhancing bone apposition in right hands while left hand dominance will reduce it by enhancing bone apposition on the left hand.

Significance to Biomedical Research and the Program of the Insitute: Dermatoglyphics are valuable genetic markers utilized in investigating the etiology of clinical entities. Dermatoglyphics are genetically determined, mostly through multiple genes. They are nevertheless vulnerable to intrauterine disturbances up to the time of their final development. Once development is completed between the 3rd and 4th month of pregnancy, dermatoglyphics remained unchanged (except for size) throughout the individual's life time. Dermatoglyphics, therefore, reflect effects of genetic anomalies, diseases or intrauterine disturbances, affecting the embryo during the first trimester of pregnancy. A final advantage in using dermatoglyphics in research is their uniqueness. That is, no two individuals (including identical twins) have identical dermatoglyphics.

Handedness and lateral dominance in general are easily identifiable manifestations of cerebral dominance. The results of the lateral dominance tests provide additional genetic polymorphic data, contribute to our efforts to understand normal anatomical asymmetry and may offer clues into the etiology of anomalies demonstrating bilateral asymmetry or unilateral occurrence.

Proposed Course: To continue the analysis of the collected data and publish the results. To expand the study of possible associations between dermatoglyphics and breast cancer and diabetes in order to confirm the preliminary findings. To ascertain the dermatoglyphic frequencies of coronary heart disease and cancer of all types among BLSA participants as well as among participants of collaborative units. To continue the population and genetic dermatoglyphic analysis for characterization of appropriate control samples. To finish the analysis of the lateral dominance and visual function data of the BLSA participants and prepare reports for publication.

Publications:

Plato., CC., Garruto, R.M. and Newman, M.T.: Total and lateral and a-b palmar interdigital ridge counts among Northern and Southern Peruvian Quechua. Human Biology. 52: 639-650, 1980.

Wertelecki, W. Plato, C. and Plato, C.C.: Absent d triradius and dotting of the ridges in siblings. Human Heredity. 30: 368-371, 1980.

Plato, C.C.: The worldwide distribution of dermatoglyphics. Proceedings of the International Symposium on Dermatoglyphics, Patiala, India, Feb., 1980. In press

Ahuja, Y., Plato, C.C., Igbad, M.A. and Sahay, B.K.: Dermatoglyphics of diabetes mellitus: revisited. Proceedings of All Indian Seminar on Human Variation, Patiala, India. In press.

Hoff, C., Plato, C.C., Garruto, R.M. and J. Dutt: Dermatoglyphic assessment of the genetic relationships of native American populations. Am. J. Phy. Anthrop. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00022-05 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Investigations of Osteoarthritis and Bone Loss

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
	A.H. Norris	Chief, Human Performance Section	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous Systems Studies	CNS NINCDS
	R.M. Garruto	Sr. Staff Associate	CNS NINCDS
	W.W. Greulich	Dept. of Anatomy, Stanford U.	
	R.T. Yanagihara	NINCDS Research Center, Guam	NINCDS
	K.M. Chen	NINCDS Research Center, Guam	NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.50	0.80

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Osteoarthritis and bone loss are the two principal age related changes of the human skeleton. Even though these changes are considered inherent to aging, they may result in incapacitating ailments. The advanced cases of osteoarthritis (degenerative joint disease) produce severe restriction of movement associated with pain. Advanced bone loss may result in osteoporosis and frequent bone fractures. Most prominent are vertebral compression fractures and fractures of the femoral neck. The following skeletal sites are involved in the present study: hand-wrist, ulna and radius and vertebral column. This project deals with the epidemiological, genetic and longitudinal aspects of osteoarthritis and bone loss among (1) the participants of the Baltimore Longitudinal Study, (2) in a sample of normal Guamanians (Chamorros), (3) among patients afflicted with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam. (4) Guamanian children of ages 7-17 who lived on Guam during periods of severe nutritional deprivation.

PHS-6040
(Rev. 2-81)

Objectives: The objectives of this study are: 1) To study the cross-sectional and longitudinal changes in bone measurements, bone mineral content and bone loss at different parts of the skeletal system of male and female participants of the BLSA and to identify factors which may affect them. 2) To evaluate the cross-sectional longitudinal and bilateral aspects of osteoarthritis in the digits and the vertebral column. 3) To compare age of onset and rate of bone loss between BLSA male and female participants and other populations. 4) To investigate the longitudinal, familial and secular changes in bone measurements and bone density by examining Guamanian x-rays of the same individuals collected 33 years apart. 5) To study a) the possible differences in bone density between patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PD) of Guam and a non-affected Guamanian sample, b) the effect of long term immobilization, due to paralysis, on bone mineral content. and c) to study the relationship between radiographical evaluation of bone mineral content and calcium metabolism assays.

Methods Employed: Bilateral radiographs of the hand and wrist from BLSA participants, from Guamanian patients affected with Amyotrophic Lateral Sclerosis (ALS) or Parkinsonism Dementia (PD), and from non-affected adult Guamanians evaluated for osteoarthritis and bone mineral content. All radiographs were obtained using similar film, at exposure of 1.0 second at 100 MA and 60 KVP without intensifying screens. Osteoarthritis grading of proximal, distal and metacarpophalangeal joints is carried out utilizing the internationally accepted grading system of Kellgren. Bone measurements, total width, medullary width and length, were done at the midshaft of the second metacarpal directly from the radiographs using vernier caliper. Cortical thickness was derived by subtracting medullary from the total width.

Major Findings: A) Evaluations of bilateral measurements of the second metacarpal of female BLSA participants resulted to the following conclusions (results of the study of the males were reported last year): 1) Females have shorter and smaller (less total width) second metacarpals than males of similar ages. 2) Right hands have longer and wider bones than left hands of the same individual. 3) As in the males, there is no significant change in bone total width or length after maturity is attained. 4) medullary width increases abruptly and continues to increase after the age of fifty years, resulting to significant reduction of the combined cortical thickness. The relationship of menopause to this bone loss is under investigation. 5) Females lose more bone with age and at a higher rate than males. The average combined cortical thickness of an 85 year old BLSA female participant is about 63% of that of a 25 year old female participant. The 85 year old male participant has 85% of the bone of a 25 year old male. B) Comparative measurements of the second metacarpal of (individually matched for age and sex) BLSA caucasian participants and normal Guamanians (females also matched for menopause status) showed that male and female BLSA participants have longer and wider second metacarpals than Guamanians of the same age and sex. The medullary width of the two groups (BLSA and Guam) was not significantly different. C) Guamanian children of ages 7-17 were also shown to have smaller (less total width) and shorter second metacarpals than white, black or Mexican Americans of the same ages. As in the adults, the medullary width of the four children's groups were not significantly different. Biological, and sociocultural factors suspected to be contributing to these differences between Guamanians and BLSA caucasians are now being investigated.

Significance to Bio-Medical Research and the Program of the Institute: Loss of bone tissue and deterioration of the joints are significant causes of disability and death in older people. Determination of the existence and degree of disease in relatively well ambulatory people provides a unique opportunity to compare bone loss and degeneration with other characteristics of these people. Nutrient intakes, activity levels and muscle strength among other things may be compared.

Proposed Course: To finish the evaluation of the collected data and publish the results. To continue the collection of bilateral x-rays from the male as well as from the female participants of the Baltimore Longitudinal Study. Now that the prevalence and rates of change in bone loss and joint degeneration have been described, comparisons of these processes with other characteristics and processes in these participants have been undertaken. Such things as nutrient intakes, medications, socioeconomic status, physical activity and muscle strength will be considered. To evaluate the data on the ALS/PD patients, suspected patients and non-affected Guamanian controls. To study the cross-sectional and longitudinal aspects of bone density and osteoporosis in the thoracic spine and the ulna and radius of the arm, and to compare the relative severity and rate of bone loss at different skeletal sites. To ascertain possible associations between the two types of bone changes (osteoarthritis and bone loss) and certain diseases as well as physiologic anthropometric variables. To investigate the relationship of bone loss to blood levels of calcitonin, parathyroid hormone and its subchains and other hormones, as well as vitamin D, calcium, phosphorus, fluoride and magnesium. To compare bone density values obtained through x-rays with those of other methods.

Publications:

Plato, C.C. and Norris, A.H.: Bone measurement of the second metacarpal and lateral hand dominance. Proceedings of International Symposium on Human Growth, Patiala, India. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00028-05 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Epidemiological and Genetic Studies of Amyotrophic Lateral Sclerosis/ Parkinsonism Dementia (ALS/PD) Complex of Guam

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous Systems Studies	CNS NINCDS
	R.M. Garruto	Sr. Staff Associate	CNS NINCDS
	R.T. Yanagihara	Research Associate	CNS NINCDS

COOPERATING UNITS (if any)

C & F Research Center, NINCDS

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0.40	PROFESSIONAL: 0.30	OTHER: 0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is: 1) to investigate the genetic and epidemiological factors contributing to the very high incidence of Amyotrophic Lateral Sclerosis and Parkinsonism Dementia (ALS/PD) on Guam, 2) to evaluate the distribution of the various established genetic and anthropological markers among the normal Guamanian population and compare them with those of the ALS/PD patients, and 3) to ascertain the effects of immobilization due to paralysis on bone density.

PHS-6040
(Rev. 2-81)

Objectives: Our involvement in this multidisciplinary project has the following objectives: 1) to determine the extent of genetic involvement in the etiology of amyotrophic lateral sclerosis and Parkinsonism Dementia (MLS/PD) complex of Guam. 2) to study the distribution of several established genetic markers such as blood groups, serum proteins, red cell enzymes and dermatoglyphics in the normal Guamanian (Chamorro) population to be used as controls in comparisons with patients, 3) to determine the prevalence of osteoarthritis, osteoporosis and other bone diseases in Chamorro ALS/PD patients and non-affected controls, and 4) to study bone growth and development of normal Guamanian children and adolescents in a 33 year follow-up study (See project #Z01 00022-05 CPB).

Methods: Two separate approaches were followed in our efforts to ascertain the familial and genetic aspects of ALS/PD. First, the patient control registry panels (registries). The registries were initiated in 1958 and closed in 1963. At the time of their closing they consisted of 136 patients, 136 individually matched nonaffected controls and their respective first degree relatives (parents, sibs and offspring) and spouses. The objective of this approach is to determine whether first degree relatives of patients have increased risk of developing the disease than relatives of controls. This year, twenty-two years after their establishment, the data of the registries were updated. The relatives of both patients and controls who developed either disease during the past twenty-two years have been identified and were recorded in the recurrence registry. All registry members who died during the same time were identified (by cross checking with the Guam death certificates) and the date of death and cause were recorded. The registry data are now ready for final analysis. The second approach is through the Umatac pedigree and the individual patient pedigrees. The Umatac pedigree, represents an elaborate genealogy of all individuals born in Umatac or from parents who were born in Umatac, the village with the highest prevalence of ALS/PD. As in the case of the registries all members of the Umatac pedigree who developed either of the diseases were identified and recorded. Marriages, deaths and births are also being recorded on the pedigree. The passage of twenty years resulted to the increase of number of offspring at the ages of high risk since many who were in their thirties at the beginning of the study are now in their fifties. The data are now being analyzed.

Major Findings: Continuing epidemiologic and familial investigations on ALS/PD of Guam resulted to the identification of forty-five families in which both parents are affected with ALS/PD. Of these 14 couples are both affected with ALS, 11 are both affected with PD and in 20 families one parent suffered from ALS and the other from PD. This new data formed the doubly affected registry which together with the singly and none affected families of the registries, will be utilized in genetic segregation analysis. Studies on the blood group and immunoglobin allotype frequencies of ALS/PD patients and nonaffected controls were not suggestive of associations between any of these variables and these diseases. Results of dermatoglyphic comparisons between patients and controls are discussed in Project #Z01 AG 00021-17 CPB. Results of patient and control bone measurements and bone loss are discussed in Project #Z01 AG 00022-04 CPB.

Significance to Bio-Medical Research and the Program of the Institute: The ultimate goal of this multidisciplinary program is not only to elucidate the etiology of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam, but also to provide a model for studying the genetic and familial aspects of other neurological diseases and dementias which are for the most part diseases of old age. It will also provide information on the effect of immobilization due to paralysis on bone mineral content (bone loss).

Proposed Course: To analyze the twenty year follow-up data of the ALS/PD Patient Control Registry, the Double Affected Registry and Umatac Pedigrees. To complete the evaluation of bone loss data among patients with ALS/PD and non-affect Chamorros. And, to prepare reports for publication.

Publications:

Plato, C.C., Garruto, R.M., and Gajdusek, C.D., Aging and chronic degenerative diseases of the central Nervous system. II. Utilization of patient control registries in studying late onset diseases. Am. J. Phys. Anthrop. 54: 263-264, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00010-8 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Noninvasive Assessment of the Left Ventricle in Normal Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Fleg	Staff Cardiologist	CPB, NIA
	D. N. Das	Staff Fellow	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.3	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Prior echocardiographic work from our laboratory has demonstrated age-related increases in left ventricular (LV) wall thickness and diminution of mitral valve closure rate in both men and women, but no alteration in LV chamber size, ejection fraction or velocity of muscle shortening. In order to confirm these cross-sectional observations in a given individual, repeat M-mode echocardiograms are being performed in the men initially studied by our group an average of 6 years ago. Because no direct assessment of diastolic LV function was performed in our earlier studies, we are quantifying rates of LV filling and emptying at rest as well as the rates of ventricular wall thickening and thinning, employing computer digitizing techniques at the Applied Physics Laboratory (APL) of Johns Hopkins University.

Assessment of cardiac performance during exercise in normal subjects using radionuclide angiocardiography, and two-dimensional echocardiography and assessment of coronary flow via thallium scans will be discussed in an appendix to this report.

Project Description:

Objectives: M-mode echocardiography is an ideal technique for investigating cardiac structure and function. At present all of the echocardiograph age-related changes in cardiac structure and function have been described only by cross-sectional study; no longitudinal studies have been performed in normal subjects. This six-year follow-up echocardiographic will constitute the first repeated measure in a longitudinal assessment of ventricular performance. The presence of age-related increases in LV wall thickness and decrease in mitral valve closing velocity suggest that LV filling rates might decline with advancing age, thus helping to explain the age-related decline in cardiac performance. Although such measurements are not easily made manually, computer digitization of the echogram allows very accurate determination of the actual filling rates and rates of LV wall thickening and thinning.

Methods: Repeat M-mode echograms will be performed on all Baltimore Longitudinal Study (BLS) men who had interpretable studies in 1974-1976. LV wall thickness, systolic and diastolic dimensions, fractional shortening, mitral valve closure rate, plus aortic root and left atrial dimensions will be measured and compared with the subject's initial values by paired t test.

Echocardiograms previously performed on the BLS men and women will be outlined by hand and then digitized by the Applied Physics Laboratory and the results analyzed by the GRC computer facility.

Major Findings: None yet. Data analyses have not yet been performed.

Significance to Biomedical Research and the Program of the Institute: Information concerning the anatomic and functional aging changes of the normal human heart is vital to understanding the aging process of the cardiovascular system. Longitudinal assessment of such changes overcomes the biases inherent in a cross-sectional approach. Careful analysis of age-related changes in diastolic cardiac function may provide a vital link in understanding the impairment in exercise performance that occurs with advancing age.

Proposed Course: The current longitudinal echocardiographic study of normal men will be continued through the next fiscal year. Analysis of diastolic LV function in normal men and women by the Applied Physics Laboratory should be nearly complete by the end of FY 1981.

Publications:

Lakatta, E. G.: Determinants of cardiovascular performance: modification due to aging. J. Chronic Dis. 1981 (in press).

Appendix

Contract Number: N01-AG-7-2129

Contract Title: Non-invasive Assessment of Cardiac Structure and Function in Aging Men and Women

Contractor: Johns Hopkins University, Baltimore, Maryland
Investigators: G. Gerstenblith, Asst. Prof. Medicine
M. L. Weisfeldt, Dir. Div. Cardiology
J. Weiss, Asst. Prof. Medicine
L. Becker, Assoc. Prof. Medicine
E. D. Mellits, Assoc. Prof. Biostatistics

Money Allocated: $906,851.00

Objectives: Two-dimensional echocardiography is a new technical development which allows determination of cardiac anatomy and function in much the same manner as routine echocardiography, with the important addition that an entire plane of the heart can be visualized at once rather than a simple "ice pick" view, allowing greater accuracy in the determination of heart chamber shape, size and function. The initial goal is to examine subjects from the Baltimore Longitudinal Study over a five year period (ages 18-95) during rest and maximal semi-supine bicycle exercise to determine age-related differences in regional and global myocardial function.

Beginning in the current (fourth) year of the contract (FY 81) multi gated acquisition MUGA scans will be obtained both at rest and during exercise in subjects of all ages. This newly developed technique, which utilizes ^{99m}-Technetium as a tracer provides as much information during exercise as the two-dimensional echocardiogram, but has the distinct advantage that the yield of technically satisfactory studies approaches 100% (vs 15%) overall for the ultrasound technique.

Thallium^{201} myocardium imaging allows non-invasive assessment of regional left ventricular blood flow and will be used to determine the incidence, severity and prognostic implications of ischemic heart disease in Baltimore Longitudinal Study participants. The predictive values of this technique will be compared to that of stress electrocardiography and two-dimensional echocardiography.

Also beginning in the current fiscal year a statistical analysis of the data obtained with the thallium perfusion scans regarding the influence of age on the development of ischemic heart disease was initiated. The specific goals of this analysis are: (1) to determine the prevalence, severity, and prognostic implications of ischemic heart disease in a normal aging population; (2) to determine whether age-related changes occur in myocardial performance or structure and whether these changes are related to alterations in myocardial perfusion; (3) to determine the predictive value of electrocardiography, thallium^{201} perfusion imaging and echocardiography during exercise stress with regard to subsequent events; and (4) to relate the presence of evidence of ischemic heart disease to the presence of specific or potential risk factors documented over a 20 year period in the study population by various investigators.

Major Findings: To date approximately 450 subjects have undergone two-dimensional echocardiography. Analysis of the resting echocardiograms is in progress. Exercise tracings have been difficult to obtain, especially in elderly subjects. We have compared the echocardiographic responses to supine bicycle exercise in young subjects (mean age 31±1 yr) versus old subjects (mean age 64±3 yr). Although echo indices of end-diastolic area (EDA) and end-systolic area (ESA) did not differ between the two groups at rest, EDA increased in the old group during exercise whereas it remained unchanged in the young. ESA, however, decreased in the young during exercise but was unchanged in the elderly. These findings indicate distinct age-related differences in the mechanisms of increasing stroke volume during exercise.

Thus far data from 27 subjects who have had stress MUGA has been analyzed. Preliminary results suggest that older subjects are not able to increase their ejection fraction during exercise to the same extent as younger ones. The data also appear to confirm our two-dimensional echocardiographic findings that older subjects increase stroke volume during exercise primarily through a Frank-Starling mechanism whereas younger subjects do so by decreasing end-systolic volume.

Nearly 350 subjects have received exercise thallium^{201} scintigrams. Thallium scanning has shown an increasing rate of positivity with age, even in asymptomatic subjects. We define latent coronary heart disease (CHD) only when both the exercise ECG and thallium scan are abnormal. Using these stress criteria (SC) and the standard resting criteria (RC) for CHD of angina, myocardial infarction by history or abnormal Q waves on standard ECG, we have defined the prevalence of CHD in the table below.

Age (yr)	40's	50's	60's	70's	80's
N	41	70	73	36	10
RC	0%	13%	15%	22%	20%
RC+SC	0%	24%	37%	56%	50%

These combined figures (RC+SC) agree closely with the prevalence of CHD found in autopsy studies and suggest that stress thallium scintigraphy may provide a useful tool to enhance the detection of CHD, thus providing the epidemiological aspects BLSA with a unique feature.

Significance to Biomedical Research and the Program of the Institute: Resting two-dimensional echocardiography and MUGA scans allow detailed non-invasive analysis of cardiac structure and ventricular function and should expand our findings from M-mode echocardiography. These techniques have helped to elucidate the mechanisms for the diminution in maximal cardiac performance with age and to detect early pathological changes in cardiac muscle function.

Thallium^{201} myocardial imaging permits the non-invasive detection of coronary heart disease in an asymptomatic population. The combination of MUGA, exercise electrocardiography, and thallium scanning represents a new epidemiologic approach to the detection of this disease--the major cause of death among the elderly. The diagnosis of latent coronary artery disease should allow further insights into the natural history and the effects of various therapeutic interventions on the disease process.

Proposed Course: Currently the contract is in the fourth of its scheduled five years. During the remainder of this fiscal year and next, additional thallium and MUGA studies will be performed. Concomitantly, all data will be analyzed with regard to the effect of age on parameters measured. In addition, the multivariate statistical analysis of the significance of risk factors data gathered by various investigators of the Baltimore Longitudinal Study of Aging will be implemented.

Staff Assessment: This contract is a direct extension of intramural research projects. Cardiovascular Section Chief, who is the Project Officer, and other members of the Cardiovascular Section have been involved in the conceptualization and design of this research. Over all the contract has been successful in meeting the objectives as set forth. It is anticipated that both the targeted number of studies as well as data analysis will be complete at the projected termination date of the contract, i.e., September 30, 1982. Longitudinal assessment of cardiac blood flow and function during stress is being contemplated. Whether it will be feasible to do this testing within the scope of the intramural program or via contract mechanism is also being considered.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00029-04 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Efficacy of Digitalis in Patients with Stable Congestive Heart Failure and in Normal Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Fleg	Staff Cardiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Mayland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies comprised two parts. (1) A double-blind crossover study of digitalis in patients with stable congestive heart failure (CHF) and sinus rhythm showed that digitalis could be discontinued for three months without adverse clinical effect and only a 3 per cent increase in resting heart size and 10 per cent decline in velocity of fiber shortening. The new dimension to this aspect of the study is that long-term follow-up (11-33 mo) of these patients has verified our favorable initial experience in discontinuing digitalis. (2) We have recently compared the effects of a digitalis intravenous bolus in healthy young and elderly men, employing systolic time intervals as a monitor of the inotropic effect and have found no age effect.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Digitalis glycosides ranked fourth in a recent audit of the most frequently prescribed drugs. Given the facts that the incidence of CHF rises expontially with age, that 11 per cent of our population is currently over age 65, and that the incidence of digitalis toxicity is highest in elderly subjects, it becomes important to demonstrate that older subjects derive as much inotropic benefit from the drug as do younger subjects. In fact data from our laboratories in intact dogs and isolated rat cardiac muscle suggest that older animals do not respond as fully to glycosides as do younger ones. We therefore compared the inotropic response to a bolus of intravenous ouabain, a rapid-acting digitalis preparation, in normal young and old men.

Methods: Healthy men from the Baltimore Longitudinal Study (BLS) under the age of 40 or older than 65 years were recruited. All subjects were free of heart and lung disease by history, physical examination, chest x-ray, pulmonary function testing, and electrocardiography at rest and during maximal exercise. No subjects were taking cardioactive medications. After giving informed consent, three sets of systolic time intervals (STI) were obtained 10 minutes apart. The men then received 0.15 mg/kg propranolol given intravenously over 10 minutes, in order to produce complete beta-adrenergic blockade. [Previous work by Daggett and Weisfeldt has shown that the effects of digitalis in normal animals may be masked by reflex adrenergic withdrawal.] Additional STI were recorded 5 and 15 minutes after propranolol infusion. Next, a 0.5 mg bolus of ouabain was administered intravenously and STI recorded at 10, 20, 40 and 60 minutes after infusion. Blood pressure was recorded simultaneous with every STI measurement.

Major Findings: Preliminary analysis shows no significant differences in digitalis responsiveness in the two age groups. In each group, the electromechanical systole was shortened approximately 4.5 per cent by ouabain and the rate of pre-ejection period to ejection time was reduced to a similar extent.

Significance to Biomedical Research and the Program of the Institute: In humans, advanced age per se should not decrease the expectation of an inotropic response when glycosides are given acutely.

Proposed Course: Further studies on the efficacy of digitalis in subjects with stable CHf during exercise are planned. These will involve the effect of digitalis on maximal oxygen consumption as well as heart size and function during maximal exercise. The results as described in Part (1) in the summary have been submitted for publication in JAMA.

Publications:

Fleg, J. L., and Lakatta, E. G.: Long-term digitalis therapy in heart failure (Letter). New Engl. J. Med. 304: 1044, 1981.

Lakatta, E. G., and Gerstenblith, G.: Cardiovascular gerontology. In: J. W. Rowe (Editor), Textbook on Geriatric Medicine. Little Brown and Co., Boston, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00030-03 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Altered Contractile and Mechanical Properties in Senescent Model Tissues: Rat Heart; Dog Aortas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Spurgeon	Physiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	E. S. Beard	Chemist	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)
F.C.P. Yin, Div. Cardiology, Depart. Medicine, Johns Hopkins Medical Institutions

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Increased dynamic stiffness across the frequency range from 35 to 0.5 Hz in maximally activated strips of aorta obtained from senescent beagles as compared to adults was demonstrated. These changes accompany changes in intrinsic impedance measured in the same animals prior to sacrifice. Dynamic stiffness measured during a cardiac contraction in rat cardiac muscle has been shown to be dependent on the degree of activation produced by adjustment of bathing [Ca^{++}], and implies that stiffness measured thusly is in part directly related to the activation process.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Several concurrent programs of an ongoing nature are associated with this project. All are designed to measure the changes in physical properties accompanying the aging process in cardiovascular tissues. These components may be broadly classified as (1) myocardial: stiffness in the isometric rat cardiac muscle obtained from young and senescent rats, and (2) vascular: circumferential stiffness [or the stress/strain] in adult and senescent dog aortas. The latter is part of a collaborative study with Johns Hopkins Division of Cardiology. The former is an evaluation of the inotropic responsiveness and stiffness correlates of rat cardiac muscle. Each attempts to relate the physiological performance with changes in the physical characteristics of the tissue, and its responsiveness to alterations in the inotropic status produced by changes in ionic environment.

Methods:

Project (1)

This portion of the study of physical correlates of cardiac function is an extension of previous investigation regarding the role of myocardial stiffness in the contractile process as it relates to hypertrophy. As reported last year, the age associated hypertrophy and concomitant stiffness changes could be in part accounted for by afterload induced hypertrophy in adult animals. The relationship of stiffness and activation, however, was not clear. An attempt has therefore been made to investigate the relative role of inotropic activation in modulating the stiffness of cardiac muscle. Substances known to produce changes in inotropy such as changes in the bathing [Ca^{++}] or the addition of the neurohumoral analog Isoproterenol were employed across a range of activations from threshold to maximum. Dynamic stiffness at 35 Hz was computed during stimulus induced isometric contraction at 25 BPM.

Project (2)

Aged beagle dogs from GRC colonies, after having been studied for dynamic impedance, were sacrificed and circumferential strips from the proximal and distal thoracic aorta removed. These strips were then mounted in a special myograph, equilibrated, and maximally activated by epinephrine. Frequency response curves at discrete frequencies of sinusoidal forcing function from 35 to 0.05 Hz across specimen lengths from slack length to 135% of slack length were determined. Thus both phase and intrinsic stiffness [or stress/strain] could be calculated. Since under maximal activation, components of both vascular smooth muscle and connective tissue are present, the tissue was then metabolically poisoned and the measurements repeated. Thus, the contribution of smooth muscle could be evaluated as the difference between the two functions produced.

Major Findings:

Project (1)

Reductions in both tension development and dynamic stiffness were found as calcium in the bathing medium was reduced. This is supportive of the concept of stiffness being an active process correlating to activation, and allows the use of stiffness as a tool to investigate the changes in activation accompanying advancing age.

Project (2)

Both vascular impedance and aortic stiffness are increased in the old by approximately equal amounts. The entire frequency response curve seems shifted up in the senescent relative to the adult animals. The increase in diameter of the aorta which accompanies senescence also approximates the magnitude of change seen in stiffness, producing only a small reduction in the overall cardiac load. These opposite changes are probably compensatory in nature.

Significance to Biomedical Research and Program of the Institute: Little previous data exists on direct measures of stiffness of the aorta as a function of age. Most other data relates to the passive component of stiffness in autopsy material, and does not address the active and dynamic nature of the tissue. The relatively complete characterization of the frequency components of the aorta at physiologic lengths and under near physiologic conditions is important to our understanding of the physiology of the aged vascular system.

Further evidence of the process of activation in cardiac muscle produced by inotropic agents is produced by the first project. The demonstration of the dependence of stiffness on the activation process, which has been previously shown to also be dependent on age, is another step in the understanding of the interrelation of calcium, advancing age, and decreased cardiovascular function.

Proposed Course: Damage at the mounting ends of the stiffness preparation remains a concern, and makes the interpretation less clean than we would like. Precision optical tracking of the length changes produced by the sinusodial forcing function will help to eliminate this difficulty and may be responsible for the remainder of stiffness changes associated with age which currently cannot be accounted for.

Publications:

Guarnieri, T., Filburn, C. R., Zitnik, G., Roth, G. S., and Lakatta, E. G.: Contractile and biochemical correlates of β-adrenergic stimulation of the aged heart. Amer. J. Physiol. 239(Heart Circ. Physiol. 8): H501-H508, 1980.

Lakatta, E. G.: Effects of advanced age on the cardiovascular system. Amer. J. Physiol., "Invited Reviews" Series. (in preparation).

Lakatta, E. G.: Age-related alterations in the cardiovascular response to adrenergic mediated stress. Fed. Proc. 39: 3173-3177, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00032-03 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Contractile, Biochemical and Electrical Catecholamine Responses in Hyperthyroid Heart

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	H. A. Spurgeon	Physiologist	CPB, NIA
	E. S. Beard	Chemist	CPB, NIA

Other: None

COOPERATING UNITS (if any)

J. Y. Wei, Beth Israel Hospital, Boston, MA

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Changes that occur within the cardiac cell in response to β-adrenergic stimulation result in a dual effect on the mammalian cardiac contraction: a potentiating effect, manifest as an enhancement of twitch force, and a relaxant effect, evidenced by a decrease in the time to peak force and twitch duration, and an enhanced rate of relaxation. Although the relaxant effects can occur in the absence of twitch potentiation, it is not known whether the cellular events that result in the relaxant effect are a prerequisite for twitch potentiation in mammalian cardiac muscle. One approach toward an understanding of this would be to selectively block the relaxant effect of β-adrenergic stimulation. We and others have demonstrated a reduction in the cAMP-dependent protein kinase-mediated stimulation of the rate of calcium accumulation in sarcoplasmic reticulum isolated from the hyperthyroid heart compared to euthyroid controls. In as much as increased rate of calcium accumulation by SR is a potential mechanism for the cAMP-mediated relaxant effect, these findings suggested to us that the hyperthyroid heart may provide a useful model in which the twitch potentiation and changes in transmembrane ionic flux in response to β-adrenergic stimulation might be dissociated from the usually concomitant relaxant effect.

Project Description:

Objectives: To determine to what extent the positive inotropic and transmembrane action potential effects of catecholamines can occur in the absence of a relaxant effect, i.e., shortening of the time to peak force in mammalian muscle.

Methods: In papillary muscles isolated from both hyperthyroid and euthyroid rats, isometric twitch force, time to peak force, time to half maximal relaxation, the time constant of force decline of the terminal portion of the twitch, and transmembrane action potential parameters that reflect slow inward current were studied across a range of isoproterenol concentrations from threshold to those eliciting maximum twitch potentiation.

Major Findings: In the hyperthyroid heart the prominent effect of isoproterenol to reduce time to peak tension (TPT) and time to half dissipation of tension (CD) that usually accompanies potentiation of twitch force by catecholamines was absent, not only at threshold but over the entire range of concentrations of isoproterenol, including those at which maximum potentiation occurred. During maximum isoproterenol stimulation, which elicited a two-fold increase in force development in both hyperthyroid and euthyroid muscles, TPT shortened 22.1±4.29 msec in euthyroid ($P<.001$) and 1.17±1.96 (N.S.) in hyperthyroid. In contrast to TPT and CD which are measured over an earlier part of the twitch, a time constant of relaxation, measured later in the twitch (peak negative dF/dt to 90% dissipation of twitch force) was reduced by isoproterenol in both hyperthyroid and euthyroid muscles. These results have been presented at the Biophysical Society Meeting in 1980 (Federation Proc. 39: (6), 2075, 1980) and a manuscript for publication is in process.

Significance to Biomedical Research and the Program of the Institute: The results indicate that the prominent shortening of TPT and CD is not prerequisite to catecholamine potentiation of twitch force and also suggest that factors governing the relaxation rate late in the twitch may differ from those that regulate TPT and CD. Thus, the results provide evidence for potentially independent effects of cAMP within the cardiac cell and indicate that changes in cellular processes which alter relaxation are not a prerequisite for potentiation in the force of contraction by catecholamines.

Proposed Course: To investigate the effects of catecholamines on ion flux and membrane phosphorylation and methylation in cell cultures treated with Tyroid hormone.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00033-03 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Ambulatory Electrocardiography and Blood Pressure Measurement in Normal Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Fleg	Staff Cardiologist	CPB, NIA
Other:	D. N. Das	Staff Fellow	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA. NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.4	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Initial ambulatory electrocardiographic work from this laboratory has characterized the normal heart rhythm patterns in healthy elderly subjects. We have extended these efforts to include younger men and women (ages 25-60). In addition, we have added a new dimension --24 hour ambulatory blood pressure (BP) recording--simultaneous with the ambulatory ECG recording, in normal subjects as well as hypertensives and those with congestive heart failure.

Project Description:

Objectives: Blood pressure (BP) is a major risk factor for the development of coronary artery disease, heart and kidney failure and stroke. Although it is widely appreciated that BP is very labile in all individuals, changing with time of day, body position, activity level and emotional state, only recently has the technology become available to noninvasively record BP automatically over a 24-hour period. Our current efforts are directed twoard characterizing the patterns of BP over a "typical" 24-hour cycle in healthy subjects over a broad age range. The technique will also be extended to hypertensives and patients with congestive heart failure.

Methods: Normal men and women from the Baltimore Longitudinal Study (BLS) will be studied across a broad age range. Subjects with known hypertension or a BP greater than 160/95 and subjects with heart failure will be studied but analyzed separately from the normals. In the normal subjects, heart and lung disease will be excluded by history and physical exam, resting ECG, maximal treadmill exercise testing, chest x-ray and pulmonary function tests.

A small 2-channel Holter recorder identical to that used in our initial study will be employed. The subject will also wear a blood pressure cuff on his non-dominant arm. The cuff will be attached to a 4 lb. metal box housing an air compressor which will inflate the cuff every 7.5 minutes and record the BP in digital form on the ambulatory ECG tape. The subject will wear the ECG and BP units for 24 hours, while undergoing the standard BLS testing protocols. He will keep a diary of all activities and symptoms over this period. The tapes will be analyzed by Dr. Harold Kennedy at Memorial Hospital in Long Beach, California using a high speed playback system plus manual validation.

Major Findings: Supraventricular and ventricular ectopy occur in the majority of a healthy active geriatric population. High degree AV conduction disturbances, profound sinus bradycardia and ischemic repolarization patterns occur frequently in normal elderly subjects. Twenty-four-hour ambulatory electrocardiography is significantly more sensitive than maximal treadmill exercise for detecting most types of arrhythmias in our population. Initial data on heart rhythm in younger adults and on blood pressure in all subjects currently being collected.

Significance to Biomedical Research and the Program of the Institute: Characterization of normal BP and heart rhythm patterns over a broad age range will allow a greater understanding of blood pressure regulation mechanisms and create a standard to which subjects with various disease processes may be compared. It will also enable more rational timing of antihypertensive and anti-arrhythmic drugs and help to separate various subsets of hypertensive subjects and define their natural history and benefit from treatment.

Proposed Course: To continue collecting data until a sufficient number of subjects have been monitored.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00034-02 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Electro-Mechanical Coupling in Cardiac Muscle from Adult and Senescent Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	H. A. Spurgeon	Physiologist	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although it is well established that certain changes occur in the cardiac contraction in muscle from senescent rats, simultaneous measurements of ventricular transmembrane action potential and muscle contraction have not been investigated. Such measurements are important since contraction is modulated by events that occur during the excitation of the muscle.

Combined into Project Z01 AG 00030-03 CPB.

PHS-6040
(Rev. 2-81)

Project Description:

Combined into Project Z01 AG 00030-03 CPB.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00035-02 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Fluctuations in the Intensity of Light Scattered through Diastolic Cardiac Muscle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	K. P. Brin	Staff Fellow	CPB, NIA
	A. A. Kort	Clinical Associate	CPB, NIA
	G. M. Bhatnagar	IPA Research Scientist	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)

M. Stern, Div. of Cardiology, Johns Hopkins Medical Institutions

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.7	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have discovered that scattered light intensity fluctuations (SLIF) are present in isolated cardiac muscle even under conditions formerly considered to be quiescent. Subsequent experiments indicated that SLIF is highly dependent on Ca^{2+} loading of the cell and could be reversibly terminated (1) by maintaining constant [Ca^{2+}] in the myofilament space in skinned fibers or (2) in intact fibers by caffeine. These results were interpreted to indicate that cellular myoplasmic [Ca^{2+}] oscillates in diastole, producing motion of the myofilaments, which modulates the laser beam resulting in SLIF. In addition to producing SLIF, this myofilament motion which is asynchronous within a cell, and among cells, results in a small degree of diastolic force or "tone" in the muscle. In additional experiments we have examined the relationship of the SLIF frequency and force development in response to subsequent excitation and have found very high correlations. Thus, the oscillatory Ca^{2+} pool monitored by SLIF appears to be intimately related to excitation-contraction coupling in muscle.

Project Description:

Objectives: (1) To determine whether SLIF are an intrinsic property of Ca^{2+}-activated myofilaments or derive their Ca dependence from oscillations in $[Ca^{2+}]$. (2) To determine whether diastolic SLIF are related to the inotropic state in cardiac muscle.

Methods: A 5 mm Helium-Neon laser is passed through right ventricular papillary muscles isolated from the rat. The muscle is isometric and the bathing fluid is maintained at 29°C. Experimental conditions are varied, i.e., the rate and pattern of stimulation are changed, $[Ca^{2+}]$ in the bathing fluid is altered, and/or pharmacologic agents known to alter the contractile state are added to the bathing fluid. Under these varying conditions, the scattered light, measured at 30° from the incident laser beam is collimated through a double pinhole system onto a photomultiplier tube. The signal is then coupled to a digital autocorrelator. The delay time of the autocorrelator function is inversely related to the frequency of intensity fluctuations of the input signal and hence provides a quantitative measure of these fluctuations. With the current methodology these measurements are made while the muscle is in the diastolic period (i.e., unstimulated state or during the period between beats at a low frequency). In other experiments, the muscle is "chemically skinned" using the non ionic detergent Triton X (1%), and $[Ca^{2+}]$, buffered by EGTA, is varied in several steps over the range of pCa that results in full activation of the muscle.

Major Findings: (1) SLIF are absent when myofilaments are activated with Ca^{2+} that results in force when the $[Ca^{2+}]$ is buffered constant. (2) SLIF when present in intact muscle are reversibly abolished by 10 mM caffeine. (3) There is a very strong correlation between inotropic state of the muscle and SLIF measured prior to excitation. Maximum potentiation of twitch force in response to inotropic interventions, i.e., increased $[Ca^{2+}]_e$, cardiac glycosides, or changes in the pattern of prior stimulation were associated with an optimal level of SLIF. When higher levels of SLIF were produced by more intense inotropic intervention, twitch force declined. Over the range of inotropic interventions up to and including that at which maximum twitch potentiation occurred, the increase in diastolic SLIF predicted the extent of twitch potentiation with a high degree of accuracy (r >0.97) both in the transient and steady states. In contrast to the other inotorpic interventions studied, catecholamines were unique in that neither SLIF nor resting force increased over a full range of concentrations that resulted in maximum potentiation of the twitch.

Significance to Biomedical Research and the Program of the Institute: The results for the first time demonstrate that during diastole isolated cardiac muscle is not quiescent. This in itself is of fundamental importance in modeling the muscle contraction and stiffness. The most recent results suggest that oscillations of cellular $[Ca^{2+}]$ occur in diastole and are the ultimate cause of SLIF. Furthermore, since SLIF increase under conditions in which the capacity to develop force on excitation is also increased, the results suggest that the oscillatory Ca^{2+} pool monitored by SLIF may be intimately related to excitation-contraction coupling in cardiac muscle. Currently no other technique is available to monitor this oscillatory Ca^{2+} pool. The results indicate that information regarding the nature of diastolic cardiac muscle can be obtained from measurements of fluctuations in light scattered through the muscle.

Proposed Course: (1) To determine whether diastolic Ca^{2+} oscillations are a universal property of cardiac excitable contractile tissues, i.e., the studies will be extended to additional species and tissues from conduction system and atrial muscle as well as ventricular muscle; (2) to time gate the cardiac cycle to determine whether rapid changes occur in SLIF in the very early part of diastole and whether these vary with the inotropic state of the tissue.

Publications:

Lakatta, E. G., and Lappe, D. L.: Fluctuations in scattered light prior to excitation predict twitch force strength in mammalian cardiac muscle. J. Physiol. (London.), 315: June 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00036-01 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Interaction of Age and Physical Conditioning Variables on Myocardial Contractility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Spurgeon	Physiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	E. S. Beard	Chemist	CPB, NIA
Other:	M. S. Steinbach	Biologist	CPB, NIA

COOPERATING UNITS (if any)
F. C. P. Yin, Div. Cardiology, Dept. Medicine, Johns Hopkins Medical Institutions

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 2.0	PROFESSIONAL: 0.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As previously reported, physical conditioning appears to be effective in modifying myocardial performance in the senescent heart. Because of the obvious importance of this area, we have undertaken to development of exercise models, varying age, duration, and intensity and type of exercise.

Project Description:

Objectives: An exercise protocol was begun to determine if the effects of conditioning could be measured in the isolated heart preparation when conditioning was begun in adults. A second series of experiments examines the effects of short term conditioning in neonates. A third series of experiments were begun to determine if forced exercise begun before development is complete (5 weeks old) and carried out on a continuing basis throughout the life of the animal would attenuate or prevent the accepted cardiovascular changes accompanying senescence.

Methods: For the first and second series chronic daily forced slow speed wheel exercise alternating with forced swimming in rats was followed by study of isometric performance. Rats were introduced in groups as neonates up to 6 weeks of age or at 5 months of age. Hearts were studied as isolated perfused septa under a variety of conditions known to alter contractile state, and compared to age matched controls. For the third series, the method chosen was to swim rats in warm (35°C) water twice daily for 20-30 minutes per session. Using a large tank with a large number of rats interacting produces vigorous exertion and near exhaustion in about 20 minutes. Weekly weight gain curves are maintained, and mortalities are autopsied for determination of heart size. The protocol has been designed to introduce a set number of 5 week old males into the program monthly, along with control unexercised animals.

Major Findings: In the group of adult animals, where exercise was begun at 5 months, some data is now available. The exercise levels employed were sufficient to produce a significant drop in body weight (Exercise=500±12 vs Control=627±12, $P<.001$) and a significant bradycardia (Exercise=271±3 vs Control=299±7, $P<.005$). Although conditioning as measured by the preceeding indices was accomplished, isometric performance of the isolated septa was not significantly different from control: across a range of perfusing Ca^{++} of 0.3 to 3.0 mmM; in the maximal response to isoproterenol (5X10^{-7}M); in response to paired stimulation; nor after 30 minutes of acidosis (pH 6.8). In the life long exercise group, no data is yet available, as the actual studies will not begin until the first groups are approaching senescence. It is anticipated that beginning in the late fall 1981 harvesting will begin and physiological isometric performance will be measured in animals at 6, 12, 18, and 22 months of age. The oldest group currently is 15 months old, therefore no isolated heart function data has yet been collected. However, the method appears feasible even though mortalities are relatively high.

Significance to Biomedical Research and the Program of the Institute: Because of the obvious interest in exercise, there is intrinsic value in the study outcome as it pertains to exercise. More importantly, since no real insight into whether or not the age-associated changes in the cardiovascular system exist as fixed and irreversible functions of age, a fundamentally better understanding of the process of aging should result.

Proposed Course: Because of limites space and resources, the initial male population will be carried through final study during the coming year. As space permits, and depending on the outcome, an additional study in female rats may be initiated.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00037-01 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Interaction of Advancing Age and Cardiac Hypertrophy on Cardiac Muscle Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. D. Walford	Clinical and Research Fellow, PHS	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	H. A. Spurgeon	Physiologist	CPB, NIA
	E. S. Beard	Chemist	CPB, NIA

Other: None

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

When the heart is faced with an increased work load on a chronic basis, cardiac hypertrophy results. With aging, certain changes occur in the cardiac cell which may alter the response to a chronically imposed work load, i.e., there may be an interaction of the adaptation to this stress and aging. The present studies were undertaken to determine whether such an interaction could be demonstrated. The elucidation and understanding of such an interaction is central to the well known observation that cardiac pathologic conditions that impose an increased work load become more severe with aging or the passage of time.

Project Description:

Objectives: To assess the performance of isolated cardiac muscle from animals with either chronic heart block or spontaneously occurring hypertension across a spectrum of age.

Methods: (1) Animal models. (a) Heart block (HB). Middle-aged (12-14 mo old) male Sprague-Dawley rats had HB induced by electrocautery of the A-V node using a transvenous approach under ECG guidance. The HB persisted until the animal's heart muscle was studied 4 to 12 months later. (b) Hypertension. The spontaneously hypertensive rat (SHR) and its non-hypertensive control, the Wistar-Kyoto rat (WK) were examined for heart rate (HR), and intra-arterial blood pressure (BP); heart weight (HW), and body weight (BW) were recorded at different ages before heart muscle was studied.

(2) Study of heart muscle. A thin left ventricular muscle was removed and mounted in a standard, horizontal muscle bath. The muscle was perfused with physiologic solution and oxygenated by bubbling gas through the reservoir. Temperature was maintained constant and the muscle was stimulated to contract by field electrodes at pre-set rate and voltage. The performance was then determined by a Statham strain gauge attached to one end of the muscle and recorded on a standard strip-recorder. Parameters measured were resting tension (RT), developed tension (DT) rate of tension development (dT/dt) and contraction duration (CD). Conditions were varied so that the response of the muscle could be analyzed under the influence of: (a) different muscle lengths, (b) changes in the ionic composition of the perfusate, (c) different rate and pattern of stimulation, (d) reduction of oxygen (i.e., hypoxic conditions), and (e) the addition of pharmacological agents to the perfusate.

Major Findings: (1) Heart size. (a) Heart block. Heart weight was increased 30% (P<.01) in HB over control inspite of same BW. Thus prolonged HB resulted in significant cardiac hypertrophy. (b) Hypertension. At 4 months of age there is minimal hypertrophy in the SHR even though BP is markedly elevated (153±9 mm Hg for SHR vs 118±10 mm Hg for WK). However, by 21 months of age, even though BP is nearly unchanged with further aging in both groups, cardiac hypertrophy in the SHR is significant, with heart size 50% greater even though BW is decreased in SHR. Therefore, even though hypertension is present at an early age and minimal hypertrophy is present, with advanced age the hypertrophy becomes highly significant.

(2) Heart muscle. (a) Heart block. Indices of mechanical performance are significantly altered showing depression DT and prolonged CD in HB. In addition, the response to repetitive stimulation is markedly depressed in HB. (b) Hypertension. Significant alterations in response to length changes, altered external calcium concentration, paired pacing and hypoxia were noted in the old SHR (i.e., old and hypertensive) group that were not present in the young hypertensive or old normotensive groups. Thus the interaction of age and hypertension result in significant abnormalities of mechanical performance. Preliminary results have been presented in part at the American Heart Association Scientific Sessions in Miami in November 1980 (Circulation 62: (4), III-13, 1980).

Significance to Biomedical Research and the Program of the Institute: The examination of the interaction of advancing age and disease in chronic laboratory animal models, here heart block or hypertension, helps our understanding of the basic properties of heart tissue, its modification by aging or time and to the challenge of withstanding added stress. This understanding is the basis for a rational approach to the modification of these stresses as they might exist in all mammalian species including man.

Proposed Course: To examine younger animals with similar duration of heart block and to examine other facets of cardiac muscle performance in both heart block and hypertension such as its light scattering properties and the function of its sarcoplasmic reticulum in order to determine basic mechanisms that may account for the changes observed in the contraction-relaxation cycle.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00093-09-CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Cellular Basis of Regulation of the Humoral Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. A. Nordin	Research Chemist	CPB NIA

COOPERATING UNITS (if any)
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LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Clinical Immunology Section

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NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 2.65	PROFESSIONAL: .9	OTHER: 1.75

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The regulatory mechanisms of lymphoid cells are being investigated in vitro using a T-cell dependent as well as a T-cell independent antigen. The cellular requirements and functions involved in the in vitro immune response are being established for normal adult mice. These are then being compared to those in aged mice of the same strains. The possibility that regulatory mechanisms observed in young adult mice are amplified in old mice resulting in immunosenescence is being investigated.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The goal of this project is to characterize the cells regulating the immune response by cellular elements in both young and aged mice. Efforts to determine the origin and mechanism of action of these cells are of prime interest.

Methods Employed:

(1) The *in vitro* culture techniques are routine methods except that a serum-free medium in which albumin, transferrin and lipids replace the conventionally used fetal calf serum.

(2) The carbonyl-iron treatment of spleen cells is accomplished by adding 25 mg of sterile carbonyl iron to 100 x 10^6 normal spleen cells. After a 30 minute incubation at 37° C in a 5% CO_2 environment, the iron and cells with ingested iron are removed by magnetic attraction. This process is repeated and the spleen cells free of iron-ingesting cells are used as a source of T and B lymphocytes.

(3) Peritoneal exudate cells are collected from unstimulated mice and used as a source of accessory cells. These cells are either used directly or an adherent layer prepared from them before other cell types are added. Supernatants of peritoneal cells collected 24 hours after culture initiation are used in some instances instead of the peritoneal cells.

(4) Purified populations of antigen specific T-cells originated from either spleen or lymph node cells of immunized C57Bl/6 mice. T-cell clones were established by the limiting dilution technique and expanded and maintained in serum-free medium supplemented with T-cell growth factor(s).

(5) DAGG-Ficoll is prepared by modifying Ficoll by introducing carbonyl methyl amino-ethyl groups to which is added the tri-peptide glycine-glycl-alanyl with the terminal alanine substituted with a single dinitrophenol haptenic group. The preparation used here contains 48 moles of hapten per mole of Ficoll.

Major Findings:

Previous studies concerned with the cellular aspects of the *in vitro* primary immune response to a T-independent antigen showed that the strict requirement for macrophages could be substituted for completely with a 48 hr supernatant of peritoneal cells. The factors contained in these supernatants were dependent on the presence of mercaptoethanol for the expression of activity and did not express full activity in the absence of T-cells. Further investigation indicated that the T-cells needed for the B-cell response to a T independent antigen are contained in normal nylon wool purified spleen cells. This indicated that antigen non-specific T-cells regulate the B-cell response in the *in vitro* primary immune response to T-independent antigens. Under conventional *in vitro* culture conditions i.e. high spleen cell density and fetal calf serum (FCS) supplemented medium, it is difficult to reveal this regulatory role of T-cells. Modifications of the conventional culture system included using a serum-free medium in which a combination of albumin, transferrin and lipids replace the requirement for FCS and the cell density was reduced so as not to exceed 2 x 10^5/culture. Cloned antigen-specific T-cells and their products were used instead of an undefined T-cell population to investigate the role of T-cells on the B-cell response induced by T-independent antigens.

Spleen cells of C57Bl/6 nu/nu when cultured in serum-free medium responded only poorly to DAGG-Ficoll. The addition of FCS to such cultures restored the response but also induced a significant polyclonal activation. This requirement for FCS could be completely replaced with factors derived from mitogen stimulated rat spleen cells. Such factors, thought to be the products of T-cells, when used at the appropriate concentration induced a B-cell response that was totally dependent on antigen. Cloned T-cells specific for the antigen egg albumin (EA) when added to 2 x 10^5 C57Bl/6 nu/nu spleen cells together with EA and DAGG-Ficoll were able to induce an antibody response to DAGG-Ficoll. As few as 10^3 egg albumin specific cloned T-cells added to the cultures were sufficient to maximally induce the response. Only selected clones of T-cells were active in this culture system and the ability to help the response to DAGG-Ficoll did not correlate with the helper function observed in a secondary-hapten carrier system. The reconstitution of the response was fully dependent on the presence of both antigens indicating that the responsiveness induced by egg albumin was not polyclonal. Also, the helper effect exerted by the cloned T-cells was fully dependent on the presence of I-A compatible macrophages. This effect of antigen-specific cloned T-cells could also be observed by adding the supernatant of 48 hr cultures of egg albumin-specific helper T-cells, I-A compatible macrophages and egg albumin. Unlike the I-A restriction observed in the presence of intact helper T-cells, the supernatants, containing the active factor(s) were completely I-A unrestricted. The activity of the supernatant titrated in a linear fashion and did not induce a response in the absence of DAGG-Ficoll.

A T-cell clone which produced an active supernatant was hybridized to BW 5147 cells using PEG as the fusing agent. T-cell hybridomas resulting from this cell fusion have been established and will be examined for production of factors regulating the immune response.

Significance to Biomedical Research and the Program of the Institute: The goal of this research program is to examine the cellular populations that are regulating the humoral immune response. The mechanisms by which the regulation takes place would be of significance not only to the field of immunology but would have relevance to cell biology. It is also significant to the area of immunosenescence. The decline in immunological responsiveness with age is well established but the reasons are not at all understood. The role of regulatory mechanisms in explaining the phenomena of immunosenescence may be of considerable significance.

Proposed Course: Studies concerned with the effect of cloned T-cells and their products on B-cells will be continued and expanded. The purpose of such studies will be to determine if T-cells influence the induction, proliferation and/or maturation of antigen stimulated B-cells. The large repetoire of T-cell hybridomas will be characterized to establish the conditions necessary to induce the release of soluble factors, their nature and function.

Publications:

Nordin, A. A. and Buchholz, M.: The Effect of Age on the In Vitro Immune Response of C57BL/6 Mice to a T-independent Antigen. In Segre, D. and Smith L. (Eds.), Immunollogical Aspects of Aging, Marcel Dekker, Inc., New York. (in press).

Saxena, R. K., Adler, W. H. and Nordin, A. A.: Monoclonal Anti Thy 1.2 Antibodies from Hybridoma HO13-4 Do Not React with Mouse Natural Killer Cells. Immunol. Communications (in press).

Bianchi, A. J. L., Hooijkaas, H., Benner, R., Tees, R., Nordin, A. A. and Schreier, M.: Clones of helper T-cells mediate antigen-specific, H-2 restricted DTH. Nature, 290, 62-63, 1981.

Schreier, M., Tees, R. and Nordin, A. A.: Establishment and characterization of helper T-cell clones: Their functional heterogeneity and effect on the B-cell responses to particulate, soluble and T independent antigens. In Feldmann, M. and Schreier, M. (Eds.), Lympokines, Academic Press, New York. (in press).

Nordin, A. A. and Buchholz, M. A., In vitro Antibody Response in Aging Animals. In Adler, W. H. and Nordin, A. A. (Eds.), Immunological Techniques Applied to Aging Research, CRC Press, Inc., Boca Raton, FL. pp. 99-108, 1981.

Nordin, A. A.: Aging and the Immune Response, in Immunology of the Eye Workshop II: Autoimmune Phenomena and Ocular Disorders. In, Helmsen, R. J., Suran, A., Gery, I. and Nussenblatt, R. B. (eds.), Information Retrieval Inc., Washington, D.C., Volume 2, 1981, pp. 287-296.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00094-08-CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization of Immune System of Aging Mice with Immunodeficiency

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. A. Nordin	Research Chemist	CPB NIA
Other:	A. J. Russo	Staff Fellow	CPB NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 2-81)

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AG-00095-08-CPB

PERIOD COVERED

October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Role of Cell Membrane Structures on Cellular Recognition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Adler	Medical Officer, PHS	CPB NIA
Other:	J. E. Nagel	Medical Officer, PHS	CPB NIA
	R. K. Saxena	Visiting Fellow	CPB NIA
	Q. B. Saxena	Visiting Fellow	CPB NIA
	R. Yanagihara	Clinical Associate, PHS	CPB NIA
	J. Curtis	Medical Staff Fellow	CPB NIA EOD 7/1/81
	M. A. Brock	Biologist	CPB NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.1	3.6	1.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project will define the functional capability of lymphocyte subpopulations in young and aging mice. Assays for functional ability and techniques for cellular manipulation allow for correlation of type of cell with particular function. The use of cell sorting apparatus will facilitate separation of subpopulations of cells in lymphoid tissue. The analysis of cellular function in aging animals will delineate the basis of the age associated decline in immune function.

Project Description:

Objectives: To correlate certain immunologic functions with morphologically identifiable subpopulations of immunologically active cells. The functional criteria and results will be compared to arrive at correlations in order to develop methods for diagnosing and describing immune deficiency and assigning certain predictive projections of immune function. The tests of immune functions will include responses to mitogens and antigens in in vitro culture conditions, responses to antigens and tumor cells in vitro and in vivo, and the development of immunologically competent cells in in vitro environments. These studies will give a better understanding of age-related immunodeficiency.

Methods Employed: The basis of most functional assays will be in vitro culture systems. There will be both short term for the investigation of mitogen, antigen and tumor cell killing responsiveness and a longer time for the generation of cytotoxic lymphocytes and antibody-forming cell colonies. In vivo methods will primarily be cell transfer studies and transplantation studies with syngeneic cells and tumor cells. The cells used will be from various mouse lymphoid organs from varying aged donors. The cells will be treated by physical separation methods and specific antisera to eliminate certain populations, or to quantitate various populations in their overall representation in lymphoid tissue. The use of a laser activated Cytofluorograph Cell Sorter will appropriate antisera will allow separation of distinct cell populations. The sorter would be used to define the cell types in lymphoid tissue, outline the changes that occur with age, and to selectively isolate specific subpopulations.

Major Findings: Some cells in human peripheral blood and mouse spleen have the ability to kill tumor cells in vitro without the necessity to have a preceeding immunization procedure. These cells are called natural killer (NK) cells and have been studied to determine the factors or agents which can modulate their level of activity. Various substances or antisera have been used to study the control mechanisms for the level of NK cell activity. Previously described data on the use of antisera to modulate activity have shown that in many cases the augmentation of NK activity by alloantisera is dependent upon the presence of FC receptors on the tumor target cells. It would appear that the alloantisera forms bridges between the tumor target cell and the natural killer cell leading to enhanced levels of lytic activity. The augmentation of NK activity by other substances such as interferon or presumably interferon inducers appears to be modulated through an entirely different mechanism which as of yet is not completely understood. Mitogens such as Con A may increase NK activity through several mechanisms such as interferon induction or the introduction of T cell growth factors. The use of cyclosporin A has allowed the dissection of the augmentation response in natural killer activity. Results so far show that many of the enhancers of natural killer cell appear to work at nuclear levels rather then at membrane levels whereas many of the inducers of the active substances appear first to interact with the natural killer or T cells at membrane levels. Previous studies have shown that NK activity can be influenced by diet, ethanol injestion and drugs such as cyclosporin A. Recent data show that hormone levels and vitamin ingestion could also influence NK activity. Growth hormone has been shown to have marked effects on NK activity in hypophysectomized mice. The use of genetically T cell deficient mice presumed to be also NK cell deficient has shown that in many cases the NK cell appears to have a relationship with T cells. This relationship may be that NK cells are part of a T cell precursor, or that T cells produce factors which influence NK activity. It has also been suggested that in many cases killing of tumor cells may be modulated by subpopulations of monocytes rather than natural killer cells. This is especially true in the killing of YAC tumor cells by human peripheral blood leukocytes, in which case it appears that the cells able to kill the YAC tumor cells are monocytes rather than natural killer cells. Experiments using monoclonal antibody with specificity against monocytic cells is now being used with the cell sorter to obtain a population of monocytic cells in order to study their function.

Studies of immunity of old mice to bacterial diseases has taken several different courses. Immunization of mice with pneumococcal polysaccharide demonstrated that the highest titers were obtained in mice from 12 to 18 months of age. The titers then dropped significantly in mice over 24 months of age. Titers were also low in mice under 3 months of age. When immunized mice were challenged with live phenococci the survival of the mice at 24, 48 and 72 hours was comparable in all age groups except in the mice under 8 weeks of age. Mice under 8 weeks of age had a very high mortality even though they had serum antibody levels comparable to old mice. Therefore, even though old mice make less antitype III antibody their level of protection is comparable to young adult mice. Further studies have shown that the spleen cells from young adult and old mice are roughly comparable in their ability to kill bacertia in vitro.

Antibody forming responses of old mice have been investigated to determine the level of T cell function in helping B cells manufacture antibody. In doing this, T cell factors which have an influence on T cell growth, and function have been tested in vitro. It has been found that the antibody forming response to certain antigens can be boosted in vitro by the addition of T cell growth factors to the culture media.

T cell growth factors have also been shown to have an effect on natural killer cell activity in vitro. The mechanism by which T cell growth factors are able to influence both T cells and non T cells such as natural killer cells is presently under investigation.

Significance to Biomedical Research and the Program of the Institute: The determination of the significance of relative immune deficiencies as seen in aging will be of immediate benefit in ccellular medicine. The determination of the level of immune function in both laboratory animals and humans can be translated into further studies to determine the correlation between that level of function in the assay system and the development of disease in the whole animal. The study of factors which can augment or modulate immune function of cells within the lymphoid system may allow a specific eththerapy or specific adjuvant type procedure to boost the level of immune function in those individuals identified as having significantly lower function.

Proposed Course: To outline the connection between form and function and to expand the technical ability to measure the function of immunocompetent lymphoreticular cells to develop methods and procedures by which functional ability of lymphoreticular cells can be boosted. To determine whether augmenting immunoactivity can be shown to be a significant factor in the resistance to disease.

Publications:

Saxena, R.K., Saxena, Q.B., and Adler, W.H.: Modulation of natural cytotoxicity by alloantibodies III. Augmentation of natural killer activity by

alloantisera directed against K, D, and I regions of the murine H-2 complex. Cell. Immunol. 56: 89-98, 1980.

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Saxena, R.K., and Adler, W.H.: Monoclonal anti Thy 1.2 antibodies from hybridoma H013-4 do not react with mouse natural killer cells. Immunol. Commn. 9(4): 371-378, 1980.

Adler, W.H.: The Use of the Mitogen Assay in Research on Aging and Immune Function. CRC Series in Aging: *Immunological Techniques Applied to Aging Research*, W. H. Adler and A. A. Nordin (Eds.) CRC Press, Boca Raton, FL, 1981.

Saxena, Q.B., Saxena, R.K., and Adler, W.H.: Regulation of natural killer activity *in vivo* III. Effect of hypophysectomy and growth hormone treatment on the natural killer activity of the mouse spleen cell population. Int. Arch. Allergy Appl. Immunol., in press.

Saxena, R.K., Adler, W.H., and Nordin, A.A.: Modulation of natural cytotoxicity by alloantibodies IV. A comparative study of the activation of mouse spleen cell cytotoxicity by anti H-2 antisera, interferon and mitogens. Cell. Immunol., in press.

Saxena, R.K., Spees, E.K., and Adler, W.H.: Modulation of natural cytotoxicity by alloantibodies II. Effect of anti HLA antisera on the natural killer activity of human peripheral blood leukocytes against a human and a murine cell line. Indian J. Exp. Biol., in press.

Yanagihara, R.H., and Adler, W.H.: Inhibition of mouse natural killer activity by cyclosporin A. Immunology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00096-08-CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Low Temperature Effects on Cells of Aging Individuals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I:	M. A. Brock	Research Biologist	CPB NIA
Other:	W. H. Adler	Medical Officer, PHS	CPB NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

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INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.0	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to characterize age-related differences in the in vitro responses of murine lymphohemopoietic cells to mitogens and their differential susceptibility to freezing damage. Because there are optimal cooling velocities for the cryopreservation of each cell type, T- and B-lymphocytes from young animals can be recovered with slight impairment of function using an optimal cooling rate. Changes in cellular structure and/or function with age then may be distinguishable with selective freezing injury to one or both subpopulations. Structures considered the probable sites of freezing damage are the plasma membrane and cellular membrane systems.

Project Description:

Objectives: To characterize the functional capacity and structure of pre- and post-mitotic cell types from aging individuals, specifically the possible age-related differences in susceptibility of murine and human immunocompetent cells to freezing injury. The sites of injury may be characterized with in vitro tests for structural and/or functional impairment.

Methods Employed: Cell suspensions were prepared from spleens of C57BL/6 mice. The percentage of viable lymphocytes in the unfrozen suspensions and in those that had been cryopreserved was determined using the stains, fluorescein diacetate and ethidium bromide, to identify viable and nonviable cells, respectively. Suspensions of the lymphocytes were adjusted to 15 or 30 x 10^6 cells/ml in media (RPMI-1640) containing 10% DMSO and 10% fetal calf serum. They were cooled in glass bottles at rates ranging from -0.25° to -10.0° C/min. to the low limit of -50° C using the microprocessor-controlled cooling system developed in this laboratory. After storage in the vapor phase of liquid N_2 (-196° C), the cell suspensions were rapidly thawed and the DMSO diluted before culture in vitro. The incorporation of ^{3}H-thymidine into DNA was used to assess the mitotic activity of the cells after in vitro activation of T-lymphocytes by the mitogens, phytohemagglutinin (PHA) and Concanavalin A (Con A), and B-lymphocytes by lipopolysaccharide (LPS).

Major Findings: The viability and in vitro function of mouse splenic lymphocytes were assessed in cell suspensions from young (4-6 mo. old) and older (15 and 23-26 mo. old) animals. Lymphocytes from the mice were also cryopreserved, and the effects of this stress on both viability and function were determined. As others have reported previously, B-cells responded to LPS stimulation by incorporating somewhat lower levels of tritiated thymidine and the activation of T-cells by PHA and Con A declined markedly in cells from mice in both older groups as compared to the young animals. The percentage of viable cells in all cell suspensions was similar. This data was then analyzed as a function of season of the year. It was previously reported that circannual rhythmicity was exhibited in viability and in vitro functional responses of young mouse splenic lymphocytes. The mice were housed in a constant environment where no known environmental entraining agents could synchronize the rhythms. The lowest values were reached in December-February and were followed by a spontaneous rise to peak levels in March. Peak responses to the mitogens were 2-5 times those observed at the troughs. In both of the older age groups, the amplitude of the rhythm was damped, especially that of T-cells. These findings have significant implications for immunotherapy and in the standard comparisons of immune function in individuals of different ages.

In the cryopreservation studies, it was shown previously that 60-100% of the unfrozen, control cells from young mice were recovered using cooling rates which ranged from -0.5° to -5.0° C/min. The mean recovery of viable, cryopreserved cells at the optimal cooling rate was 85.5%, and this fell to 64.3% and 51.4% for lymphocytes from 15 mo. old and 23-27 mo. old mice, respectively. Activation of cryopreserved cells from the young mice by T- and B-lymphocyte mitogens was close to control, unfrozen levels which indicates that neither subpopulation was selectively impaired by this stress. In contrast, the responses to mitogens by cryopreserved cells from both older groups of mice were markedly lower than their unfrozen controls. Interestingly, the greatest impairment of function was for B lymphocytes from the oldest animals. Whereas

50% of the functional capacity of cells from 15 mo. old mice was attained by cryopreserved cells, this was reduced to an average of only 27% in the oldest group. This reduced recovery indicates that there are marked age-related structural-functional changes which are not detected by in vitro activation of unfrozen cells with LPS.

Clear seasonal cycles in the viability and in vitro function of cryopreserved lymphocytes were also exhibited. Recovery of viable cells from the young mice declined during the autumn reaching its lowest levels ($\bar{x}$ = 50%) in January and then increasing to peak levels ($\bar{x}$ = 80%) in March-May. This corresponds in timing with the circannual rhythmicity in function of unfrozen cells. The recovery of viable cells in the two older age groups has so far not exhibited seasonal rhythmicity. The pattern of recovery of in vitro function by cells from young mice was analogous to the seasonal cycle in viability but appears to be phase advanced, that is, the lowest levels of functional activity were observed in October rather than January. Marked differences both in reduced levels of thymidine incorporation and in seasonal patterns of activation were exhibited by the cryopreserved lymphocytes from both older age groups. There appears to be selective impairment of T cell function during certain seasons, but these data are not yet complete.

Cultures of cells from all age groups, both control unfrozen and cryopreserved, were pulsed with increased concentrations of tritiated thymidine. Its incorporation into DNA was enhanced equally effectively in all cell cultures. This finding combined with the observed decline in responsiveness to mitogens 1) with age, 2) following cryopreservation of cells from older mice, and 3) seasonally suggests impairment of the activation-recognition function of the plasma membrane under those conditions.

Significance to Biomedical Research and the Program of the Institute: The reported decline in the functional capacity of lymphocytes with age may be intrinsic and/or extrinsic. These possibilities can be tested by modifying components in an in vitro system which tests functional capacity and by assessing the effects of freeze-thaw damage on lymphocytic biomembrane systems. Controlled rate cooling is a new technique that may be used to preserve lymphocytes for further study at the cellular level or for therapeutic use.

Proposed Course: Changes with age in lymphocytic resistance to the stress of cryopreservation will be continued using controlled rate cooling techniques. The characterization of age-related injury to mammalian lymphoid cells will include further studies on splenic lymphocytic responses to mitogens, particularly the cytoskeleton and biomembrane systems involved in activation of the cells.

Publications:

Brock, Mary Anne: Comparison of circasemilunar rhythmicity in east and west coast cnidarians. In: Developmental and Cellular Biology of Coelenterates. Eds: P. Tardent and R. Tardent, Elsevier/North Holland, The Netherlands, 1980, p. 23-26.

Brock, Mary Anne and Guenter Baartz: Cryoprotection of murine lymphocyte subpopulations using a microprocessor-controlled cooling system. Cryobiology, 17: 439, 1980.

Brock, M. A.; Cryopreservation of Murine Lymphocytes. In: Immunological Techniques Applied to Aging Research. Eds.: W. H. Adler and A. A. Nordin, CRC Press Inc., Boca Raton, FL, 1981, p. 215-227.

Brock, Mary Anne: Senescence in Campanularia flexuosa and other cnidarians. In: Lower Invertebrate Models for Aging Research. Ed.: D. Mitchell, CRC Press, Inc., Boca Raton, FL, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00104-05-CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Immune Survey of the Longitudinal Project Participants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Adler	Medical Officer, PHS	CPB NIA
Other:	A. A. Nordin	Research Chemist	CPB NIA FW/SP until 8/1/81
	J. E. Nagel	Medical Officer, PHS	CPB NIA
	Q. B. Saxena	Visiting Fellow	CPB NIA
	J. L. Curtis	Medical Staff Fellow	CPB NIA EOD 7/1/81

COOPERATING UNITS (if any)

None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS: 3.3	PROFESSIONAL: 1.75	OTHER: 1.55

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The immune function of an aging human population will be evaluated to determine the age associated changes and the possible effects on the incidence and types of disease. The goals are to determine which age related diseases are associated with a decrease in immune function and therefore can be modulated by augmenting the function. The accurate assessment of function in a human population is a major goal with the development of newer assays and the refinement of existing assays.

Project Description:

Objectives: The purpose of this project is to assess various human immune functions and to correlate these results into an overall profile of the individuals' immunologic abilities. Since many different assays are being performed, each is presented individually with the understanding that through correlational analysis of the data it may be possible to arrive at the most useful assays to employ in measuring human immune function.

The three broad areas of the program are data collection, research and service. The data collection and research aspects are dealt with specifically in terms of investigation of serum antibody and lymphocyte responses. The service aspect is a consultative function for other investigators wishing to utilize assays that use immunological methods, such as radioimmunoassays for hormones, fluorescent antibody staining in histopathology, and hybridoma tissue culture techniques.

The specific assays of human immune function which are used in this study are as follows.

1. The measurement of serum immunoglobulin levels by radial immunodiffusion and by the paper radioimmunosorbent test.
2. The measurement of peripheral blood granulocyte function, enzyme assays, metabolic assays and phagocytic and bacterial killing assays.
3. The identification and quantitation of lymphoid cell subpopulations in the peripheral blood.
4. Quantitation of the response of lymphocytes to cellular activators (PHA, Con A, PWM, SPA, tetanus toxoid, candida, phorbol myristate acetate).
5. Determination of the number of lymphocytes in peripheral blood which can differentiate to antibody-forming cells. There is evidence to indicate that immunoglobulin synthesis in aging mice and humans becomes restricted and often converts to a monoclonal nature. However, it is not yet appreciated if such a restriction in any way affects the integrity of the immune system.
6. The measurement of Epstein-Barr virus (EBV) infection will be determined in cells which formed lymphoblast cell lines in tissue culture. We will also determine the presence of anti-EBV antibody in the serum as evidence of exposure.
7. Measurement of serum autoantibodies. Distinct profiles of antinuclear antibodies and Rheumatoid factors are associated with certain systemic rheumatic diseases.

Methods Employed:

1. Cell Preparation:

Heparinized peripheral blood drawn from participants in the Baltimore Longitudinal Study of Aging (BLSA) will be used for cell isolation. Preliminary data obtained by flow cytometry and monoclonal antibodies (see findings) has suggested that the composition of cell populations obtained by standard cell isolation techniques such as Ficoll-Hypaque density centrifugation or dextran sedimentation are more heterogenous than previously appreciated by morphologic criteria. Although we are continuing to use these cell separation techniques, the results of the various assays are being monitored to ascertain possible effects induced by alterations in cell populations.

2. Assay Systems:

   a. Assays of immunoglobulin synthesizing cells will be accomplished by an Staphylococcal protein A plaque assay using both polyclonally (PWM) and antigen activated cells.

   b. Serum EBV titers will be determined by standard fluorescent immunochemical techniques using lymphoblast cell lines and fluorescent anti-immunoglobulin antisera.

   c. A flow cytometry system and cell sorter will be used with monoclonal antibodies to study lymphocyte subsets and other cell types.

Major Findings: A total of 743 longitudinal study participants have been studied using the various assays. To date 229 of 277 (83%) women and 514 of 756 (68%) men have been examined at least once. Eight men have been restudied 4 times; 30 men and 6 women 3 times, and 129 men and 45 women twice. Despite examining over 200 men in the past year the percentage of male participants has remained stable due to return visits, initiated through followup efforts by members of the Human Performance Section, CPB, of inactive or previously withdrawn BLSA participants.

The findings are:

1. An age related decrease in T lymphocyte proliferative responses to mitogens, however no decrease in B lymphocyte proliferation.

2. No age related changes in serum immunoglobulin levels.

3. There was no age-related decrease in natural killer (NK) activity among 200 BLSA participants. NK activity also was found not to differ between males and females or between smokers and nonsmokers.

4. Total numbers of peripheral blood T cells identified by monoclonal antibody were found to significantly diminish in elderly individuals. This absolute and relative decrease was principably accounted for by a decrease in the absolute number and percentage of T-suppressor cells. T-helper appeared to remain stable during aging.

5. The metabolic capacities of peripheral blood granulocytes evaluated by the quantitative NBT test were unchanged with age. The *in vitro* ability of granulocytes from young and old individuals to kill *Staphyloccus aureus* also was not significantly different; however superoxide production by granulocytes from elderly individuals was markedly diminished.

6. The representation of various subpopulations of T lymphocytes was found to be modified by the method of cell preparation and isolation. Flow cytometric

analysis with monoclonal antibodies indicated that approximately 10-25% of the cells obtained were monocytes rather than lymphocytes. The significance of the finding on several *in vitro* assays of T and B cell function is being evaluated.

7. Lymphoblast lines developed from the peripheral blood of several BLSA participants have now been in continuous cell culture for over 3 years. All cell lines are EBV positive and have small amounts of surface immunoglobulin associated with them. By a FITC labeled aggregated γ globulin assay all lines have Fc receptors. Several cell lines have been used as cell targets during studies of the mechanisms of murine NK activity. Three individuals have produced cell lines from blood specimens obtained 2-3 years apart. Successful cell line development may be related to the number of cells initially cultured. Serum EBV titers are higher in individuals forming lines than those of nontransformers. Possible significance of these findings is continuing to be evaluated.

8. Longitudinal analysis of the BLSA hematalogical data indicates that there are no alterations, over a 10 year time span, in the hemogram or differential leukocyte count. Twenty year longitudinal data from approximately 65 BLSA participants is presently being evaluated.

Significance to Biomedical Research and the Program of the Institute:
There is no doubt that these studies along with the other information available on BLSA participants will yield valuable data to show the presence or absence of a correlation of age-associated diseases and disorders with a deficiency of immune function. At present, there is no information on this subject. If we find that an age-associated immunodeficiency does lead to specific diseases, we could, hopefully, avoid the disorders by augmenting immune function. It may also be possible to alter immunogens to make them more effective in the aging host.

Proposed Course: A baseline of data on the immune function of the BLSA participants has now been established. Results indicate that functional declines in human immunocompetence are considerably less definitive than might be appreciated from literature reports evaluating small numbers of individuals or extrapolation from animal models. In all our population studies there are both young and old individuals who demonstrate levels of immunocompetence above and below the normal range. The significance of this finding will continue to be evaluated during follow-up visits. Comparison of BLSA immune function data with other information about participants has commenced, however no positive findings have, as yet, resulted. Increased emphasis will be placed on the evaluation of the immunocompetence of individuals with various diseases as well as the relationship of drug intake to levels of immune function. Further studies are in progress using laser activated flow cytometry to evaluate cell populations and correlate them with the immune function of BLSA participants. We will continue to restudy BLSA participants to determine the stability of immune function over time.

Publications:

Yanagihara R, Nagel JE, Adler WH: Cells of immune function *In*: Cell Biology, Vol. II, V. J. Cristafalo (ed), CRC Press, Boca Raton, 1981 (in press).

Nagel JE, Chrest FJ, and Adler WH: Human B-cell function in normal individuals of various age. I. *In vitro* enumeration of pokeweed induced peripheral blood

lymphocyte immunoglobulin synthesizing cells and the comparison of the results with numbers of peripheral B and T-cells, mitogen responses, and levels of serum immunoglobulin. Clin. Exp. Immunol. 44:646, 1981.

Saxena, QB, Mezey E, and Adler W.H: Regulation of natural killer activity *in vivo*. II. The effects of alcohol consumption on human peripheral blood natural killer activity. Int. J. Cancer 26:413, 1981.

Nagel JE, Chrest FJ, and Adler WH: Enumeration of T-lymphocyte subsets by monoclonal antibodies in young and aged humans. J. Immunol. (in press).

Nagel JE, Collins GD, and Adler WH: Spontaneous or Natural killer cytotoxicity of K562 erythroleukemic cells in normal patients. Cancer Res. 41:2284, 1981.

Office of Planning and Extramural Affairs

Program Description OPEA-1

Program Highlights OPEA-1

OFFICE OF PLANNING AND EXTRAMURAL AFFAIRS

PROGRAM DESCRIPTION

The Office of Planning and Extramural Affairs (OPEA) performs the following functions:

- Provides advice and assistance to the Institute Director and other officials on issues related to program planning and evaluation and extramural affairs;
- Provides initial scientific merit review on program projects, training grants, research contracts and other special awards e.g. Geriatric Medicine Academic Award, Small Grant Award;
- Evaluates the Institute's extramural program;
- Coordinates the Institute's training policies, procedures and programs;
- Assigns applications by program area within the Institute;
- Participates in the National Advisory Council on Aging review of applications and annual review of the Institute's programmatic activities;
- Plans, conducts and directs an array of activities in the area of program analysis, program planning and evaluation;
- Makes recommendations to senior Institute staff in planning, implementing and evaluating overall Institute programs, policies, plans and procedures;
- Provides analytical and interpretive data needed for formulating Institute goals and objectives;
- Provides information on activities of legislative committees related to Institute programs; and
- Provides liaison with other Federal agencies having research and training programs in the field of aging.

The personnel associated with OPEA is presented in Figure 1.

PROGRAM HIGHLIGHTS

Over the past year, OPEA has made substantial progress in strengthening the planning and evaluation, scientific review and program analysis functions, improving the efficiency and management of OPEA operations, and developing collaborative relationships with other Program offices. The progress made is attributable in large part to a dedicated staff of secretaries and other professionals within OPEA. Each of the three component offices of OPEA has assumed increasing responsibility for

OFFICE OF PLANNING AND EXTRAMURAL AFFAIRS ORGANIZATION

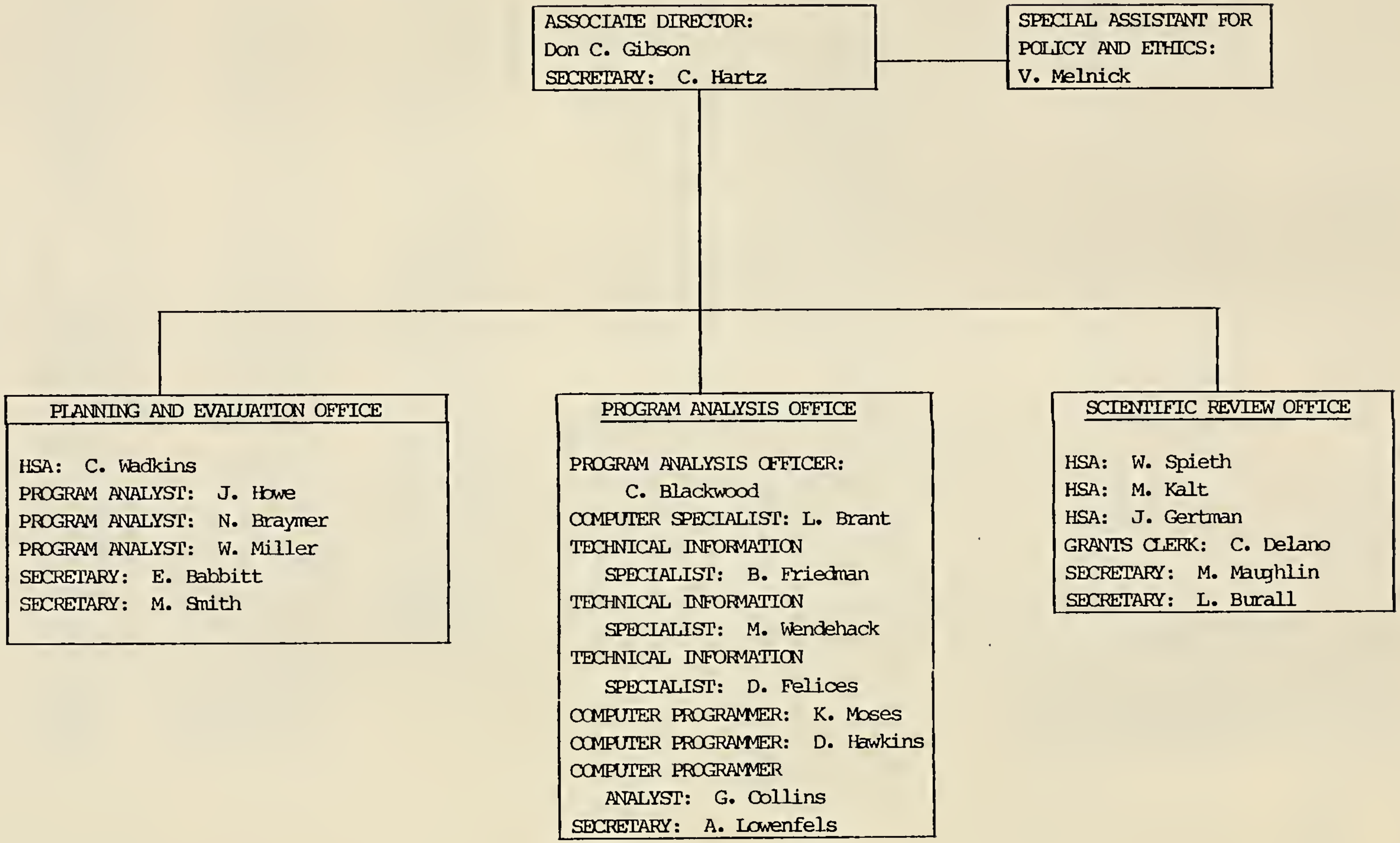

Figure 1

activities that will enhance the effectiveness of NIA and foster continued progress in the field of aging research.

In addition to conducting the internal NIA planning and evaluation cycle and coordinating NIA participation in the central NIH and Departmental planning, evaluation, and legislative processes, the Planning and Evaluation Office is currently involved in three major projects: (1) administrative and technical management of the development of a National Research Plan on Aging; (2) preparation of a comprehensive Inventory of Aging Research across the Department of Health and Human Services (DHHS), and eleven other Federal agencies; and (3) evaluation projects on the main resource programs of NIA (cells and animals) and the newly developed Geriatric Medicine Academic Award. Each of these projects requires a significant expenditure of time, effort and intellectual interchange. Also, they collectively represent a major NIA contribution to the planning, assessment and evaluation of research on aging and a context from which to address research issues of national importance at the White House Conference on Aging (WHCoA) later this year.

- The National Research Plan on Aging will be developed in three stages. An interim report on the research plan will provide the research community and WHCoA delegates with preliminary information on the plan and its significance to research on aging. This will be followed by an executive summary of the plan that may be used as the basis for identifying specific research issues, their significance, research objectives, and actions necessary to attain these. A final definitive plan will be prepared early in 1982 that addresses issues and resources required for aging research.

- The Inventory of Aging Research across DHHS and selected Federal agencies will provide a current assessment of ongoing aging research and research-related activities within the Federal establishment. This document will constitute an important instrument for effective planning of research on aging and the identification of gaps, overlaps and needs.

- The evaluation of animals and cells will provide NIA with the basic data and information needed to plan the further development of these most critical resources. Evaluation of the Geriatric Medicine Academic Award should provide the basis for decisions on how to effectively impact on scientific curriculum in medical schools and the development of clinical investigators in aging research.

Concurrently, the Planning and Evaluation Office developed the final document for the Planning/Appropriation Briefing held with the Director, NIH, and initiated the new planning cycle in preparation for the upcoming program reviews by the Director, NIA, the program planning review with the Director, NIH, and the annual October National Advisory Council on Aging review of programs.

Several important accomplishments that contribute to the effective and efficient management of Institute activities were completed and institutionalized. These include: (1) completion, in collaboration with Biomedical Research and Clinical Medicine (BRCM) and Social and Behavioral

Research (SBR) Programs, of referral guidelines for research grants that identify specific interests of NIA and overlaps with other Institutes, (2) a Contract Policy Guidelines Manual that has been adopted by the Institute, (3) a fully functioning Contract Advisory Committee that has completed contract reviews for FY 1981 and initiated those for FY 1982, and (4) development of a funding policy for research and training grants which addresses high and low program relevance determinations and type 2 competing renewals.

The Scientific Review Office provides merit review for research and training grants as well as research contracts. During the past year, this activity has increased in scope, complexity, quantity of applications reviewed, and the quality attained. The Aging Review Committee (ARC), the chartered Institute review body, was increased from 15 to 22 members. All positions are either filled or nominations have been made. This committee now reviews P01s, K08s (two types--clinical and academic), K01s and T32s. Ad hoc reviews have been conducted on a regularly scheduled basis for the Geriatric Medicine and Dentistry Academic Awards. In addition, several ad hoc reviews were conducted when an applicant was also a member of the Aging Review Committee.

The Program Analysis Office has expanded its efforts during the past fiscal year to provide program data systems and information that affects all levels of management within NIA. These activities have included: (1) implementation of a contract to develop a management information system that will describe, cluster, identify and analyze research and contract projects supported by NIA; and (2) development and operation of a fiscal ledger program to tabulate and maintain current research and contract funding commitments and plans.

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